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<input type="checkbox"/>	L1	felis and urease	137
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END OF SEARCH HISTORY

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Terms	Documents
(cs1 or cs-1 or kukka or ds4 or ds-4 or 2301 or atcc49179 or atcc-49179 or 49179) and felis	22

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Bacteria

ATCC® Number	Description	Designation	View
49179	<i>Helicobacter felis</i> Paster et al. deposited as Cat spiral 1	CS1	<input type="checkbox"/>
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Table 1b:

amino acid and nucleic acid homology between the <i>H. felis</i> ureY and various ureB subunits.		
Reference molecule : <i>H. felis</i> ureY CS1	a.a.	n.a.
<i>H. felis</i> ureB	73 %	71 %
<i>H. pylori</i> ureB	73 %	70 %
<i>H. heilmannii</i> ureB	74 %	71 %
<i>H. felis</i> strain Kukka ureY	99 %	95 %
<i>H. felis</i> strain Ds4 ureY	98 %	94 %
<i>H. felis</i> strain 2301 ureY	99 %	95 %

Table 1c:

nucleic acid homology between <i>H. felis</i> ureXY and various ureAB genes.	
Reference molecule: <i>H. felis</i> ureXY CS1	n.a.
<i>H. felis</i> ureAB	67 %
<i>H. pylori</i> ureAB	67 %
<i>H. heilmannii</i> ureAB	68 %
<i>H. felis</i> strain Kukka ureXY	94 %
<i>H. felis</i> strain Ds4 ureXY	94 %
<i>H. felis</i> strain 2301 ureXY	94 %

49179

[0006] One embodiment of the invention thus relates to nucleic acid sequences encoding the novel urease X and Y subunits.

[0007] First of all, this embodiment of the invention relates to nucleic acid sequences encoding two subunits of a urease complex such as expressed by *Helicobacter felis*, that have at least 85 % homology with SEQ ID NO: 1, or parts thereof with a length of at least 40, preferably 45, more preferably 50 nucleotides encoding at least an immunogenic fragment of one of the subunits. Still even longer fragments, with a length of at least 55, 60 or 70 nucleic acids are in that order even more preferred.

[0008] A preferred form of this embodiment relates to nucleic acid sequences that encode the urease X subunit polypeptide or the urease Y subunit polypeptide and that have at least 85 % homology with SEQ ID NO: 1, or parts thereof with a length of at least 40, preferably 45, more preferably 50 nucleotides encoding at least an immunogenic fragment of the urease X subunit polypeptide or the urease Y subunit polypeptide. Merely as an example: the nucleic acid sequence encoding the urease X subunit of *Helicobacter felis* strain CS1 starts at position 206/207/208 (GTG) (See figure 1a (1)) and stops at position 884/885/886 (TAA). the nucleic acid sequence encoding the urease Y subunit of *Helicobacter felis* strain CS1 starts at position 897/898/899 (ATG) and stops at position 2601/2602/2603 (TAG). Still even longer fragments, with a length of at least 55, 60 or 70 nucleic acids are in that order even more preferred.

[0009] A more preferred form of this embodiment relates to nucleic acid sequences having at least 90 %, preferably 94 %, more preferably 97 % homology with SEQ ID NO: 1.

[0010] The determination of the homology percentages was done with the computer program Align Plus for Windows, available from Scientific and Educational Software, P.O.Box 72045 Durham, NC 27722-2045, USA. Settings used for the nucleic acid comparisons are indicated in figures 1a, 1b and 1c.

[0011] Since the present invention discloses nucleic acid sequences encoding novel structural *Helicobacter felis* urease subunits, it is now for the first time possible to obtain such polypeptides in sufficient quantities. This can e.g. be done by using expression systems to express the genes encoding the UreX and UreY subunits.

Therefore, in a more preferred embodiment, the invention relates to DNA fragments comprising a nucleic acid sequence according to the invention. Such DNA fragments can e.g. be plasmids, into which a nucleic acid sequence according to the invention is cloned. Such DNA fragments are useful e.g. for enhancing the amount of DNA for use as a probe, as described below.

[0012] An essential requirement for the expression of the nucleic acid sequence is an adequate promoter operably linked to the nucleic acid sequence. It is obvious to those skilled in the art that the choice of a promoter extends to any



US005843460A

United States Patent [19]

Labigne et al.

[11] **Patent Number:** 5,843,460[45] **Date of Patent:** Dec. 1, 1998

- [54] **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

WOA9503824 2/1995 WIPO.
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R. L. Ferrero et al., "Molecular Evidence Demonstrating Significant Homology Between the Urease Polypeptides of *Helicobacter Felis* and *Helicobacter Pylori*," *Gastroenterology*, vol. 104, No. 4, Apr. 1993, Elsevier, New York, U.S.; p. A699.

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(List continued on next page.)

- [75] **Inventors:** Agnes Labigne, Bures S/Yvette, France; Sebastin Suerbaum, Bochum, Germany; Richard L. Ferrero, Paris; Jean-Michel Thiberge, Plaisir, both of France

- [73] **Assignees:** Institut Pasteur; Institut National de la Sante et de la Recherche Medicale, both of Paris, France

- [21] **Appl. No.:** 467,822

- [22] **Filed:** Jun. 6, 1995

Related U.S. Application Data

- [63] Continuation of Ser. No. 447,177, May 19, 1995, which is a continuation-in-part of Ser. No. 432,697, May 2, 1995.

- [30] **Foreign Application Priority Data**

May 19, 1993 [EP] European Pat. Off. 93 401 309
Nov. 19, 1993 [WO] WIPO PCT/EP93/03259

- [51] **Int. Cl.⁶** **A61K 39/02**

- [52] **U.S. Cl.** **424/234.1; 435/7.32; 435/6; 435/7.9; 514/234.5; 514/41**

- [58] **Field of Search** 435/7.32, 4, 6, 435/7.9; 514/234.5, 41; 424/234.1

- [56] **References Cited**

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WOA9320843 10/1993 WIPO.
WOA9406474 3/1994 WIPO.
WOA9409823 5/1994 WIPO.

Primary Examiner—James C. Housel

Assistant Examiner—Ginny Allen Portner

Attorney, Agent, or Firm—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

- [57] **ABSTRACT**

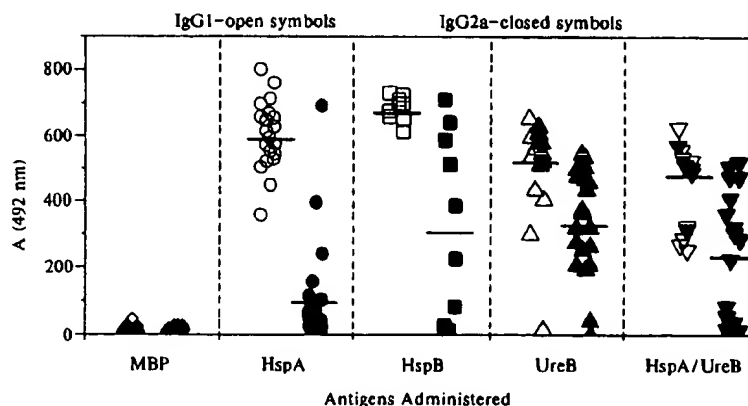
There is provided an immunogenic composition capable of inducing protective antibodies against *Helicobacter infection* characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22-26);

- ii) and/or, a heat shock protein (Hsp), or chaperonin, from *Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

10 Claims, 36 Drawing Sheets



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NCBI BLAST program reference [PMID:9254694]:

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402(1997).

Query: 243 AA (of which 10% low-complexity regions filtered out)

Date run: 2005-06-10 03:32:14 UTC+0100 on sib-gml.unil.ch

Program: NCBI BLASTP 1.5.4-Paracel [2003-06-05]

Database: EXPASY/UniProtKB

1,974,938 sequences; 640,866,274 total letters

UniProt Release 5.2 consists of: Swiss-Prot Release 47.2 of 07-Jun-2005: 184304 en

TrEMBL Release 30.2 of 07-Jun-2005: 1779481 entrie

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List of potentially matching sequences

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Db	AC	Description	Score	E-value
<input type="checkbox"/>	sp	Q08716 URE2_HELFE Urease beta subunit (EC 3.5.1.5) (Urea amid...	437	e-122
<input type="checkbox"/>	tr	Q9RGP5 _HELFE Urease (Fragment) [ureB] [Helicobacter felis]	437	e-122
<input type="checkbox"/>	tr	Q8KT24 _HELFE Urease UreB (Fragment) [ureB] [Helicobacter felis].	437	e-122
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<input type="checkbox"/>	tr	Q6UK78 _HELFE UreB (Fragment) [ureB] [Helicobacter felis]	437	e-122
<input type="checkbox"/>	tr	Q6UK66 _HELFE UreB (Fragment) [ureB] [Helicobacter felis]	437	e-122
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<input type="checkbox"/>	tr	Q8KT23 _9HELI Urease UreB (Fragment) [ureB] [Helicobacter salo...	434	e-120

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<input type="checkbox"/>	tr	<u>Q8KT25</u>	_HELBI	Urease UreB (Fragment)	[ureB]	[Helicobacter bizz...	<u>413</u>	e-114
<input type="checkbox"/>	tr	<u>Q8GH97</u>	_HELBI	Urease B		[Helicobacter bizzozeronii]	<u>413</u>	e-114
<input type="checkbox"/>	tr	<u>Q9X753</u>	_9HELI	Urease (Fragment)	[ureB]	[Helicobacter salomonis]	<u>409</u>	e-113
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<input type="checkbox"/>	tr	<u>Q64EY3</u>	_HELPI	UreB	[ureB]	[Helicobacter pylori (Campylobacter ...	<u>378</u>	e-104
<input type="checkbox"/>	tr	<u>Q9X742</u>	_HELBI	Urease (Fragment)	[ureB]	[Helicobacter bizzozero...	<u>377</u>	e-103
<input type="checkbox"/>	tr	<u>Q8KT33</u>	_HELPI	Urease UreB (Fragment)	[ureB]	[Helicobacter pylo...	<u>377</u>	e-103
<input type="checkbox"/>	tr	<u>Q8RNU6</u>	_HELPI	Urease B subunit	[ureB]	[Helicobacter pylori (Ca...	<u>376</u>	e-103
<input type="checkbox"/>	tr	<u>Q8KT30</u>	_HELHE	Urease UreB (Fragment)	[ureB]	[Helicobacter heil...	<u>376</u>	e-103
<input type="checkbox"/>	tr	<u>Q8KT11</u>	_9HELI	Urease UreB (Fragment)	[ureB]	[Helicobacter sp. ...	<u>376</u>	e-103
<input type="checkbox"/>	tr	<u>Q9AFB1</u>	_HELPI	Urease B	[ureB]	[Helicobacter pylori (Campylobacter pyl...	<u>375</u>	e-103
<input type="checkbox"/>	tr	<u>Q9S6R7</u>	_HELBI	Urease (Fragment)	[ureB]	[Helicobacter bizzozero...	<u>373</u>	e-102
<input type="checkbox"/>	tr	<u>Q6UK74</u>	_HELPI	UreB (Fragment)	[ureB]	[Helicobacter pylori (Cam...	<u>372</u>	e-102
<input type="checkbox"/>	tr	<u>Q6UK70</u>	_HELPI	UreB (Fragment)	[ureB]	[Helicobacter pylori (Cam...	<u>372</u>	e-102
<input type="checkbox"/>	tr	<u>Q8KT15</u>	_HELHE	Urease UreB (Fragment)	[ureB]	[Helicobacter heil...	<u>346</u>	2e-94
<input type="checkbox"/>	sp	<u>P50045</u>	URE2_HELMI	Urease beta subunit (EC 3.5.1.5)	(Urea amid...		<u>323</u>	1e-87
<input type="checkbox"/>	tr	<u>Q9R8R3</u>	_HELHP	Urease beta subunit (Fragment)	[ureB]	[Helicobac...	<u>319</u>	4e-86
<input type="checkbox"/>	tr	<u>Q9R8R1</u>	_HELHP	Urease beta subunit (Fragment)	[ureB]	[Helicobac...	<u>317</u>	1e-85
<input type="checkbox"/>	tr	<u>Q93PJ4</u>	_HELHP	Urease beta subunit UreB (EC 3.5.1.5)	[ureB]	[He...	<u>317</u>	2e-85
<input type="checkbox"/>	tr	<u>Q9ZHF1</u>	_HELHP	Urease beta subunit (Fragment)	[ureB]	[Helicobac...	<u>317</u>	2e-85
<input type="checkbox"/>	tr	<u>Q9RGP8</u>	_9HELI	Urease (Fragment)	[ureB]	[Flexispira rappini]	<u>312</u>	3e-84
<input type="checkbox"/>	tr	<u>Q8KT14</u>	_HELHE	Urease UreB (Fragment)	[ureB]	[Helicobacter heil...	<u>311</u>	6e-84
<input type="checkbox"/>	tr	<u>Q9RGP7</u>	_HELHP	Urease (Fragment)	[ureB]	[Helicobacter hepaticus]	<u>311</u>	1e-83

<input type="checkbox"/>	tr	<u>Q9RGP6</u>	_HELHP Urease (Fragment) [ureB] [Helicobacter hepaticus]	<u>310</u>	2e-83
<input type="checkbox"/>	tr	<u>Q9RGQ0</u>	_9HELI Urease (Fragment) [ureB] [Flexispira rappini]	<u>308</u>	5e-83
<input type="checkbox"/>	tr	<u>Q9RGP9</u>	_9HELI Urease (Fragment) [ureB] [Flexispira rappini]	<u>308</u>	5e-83
<input type="checkbox"/>	sp	<u>Q07397</u>	URE1_BACSB Urease alpha subunit (EC 3.5.1.5) (Urea ami...	<u>295</u>	4e-79
<input type="checkbox"/>	tr	<u>Q9KG59</u>	_BACHD Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacill...	<u>289</u>	3e-77
<input type="checkbox"/>	tr	<u>Q8GLB5</u>	_HELHE UreB (Fragment) [Helicobacter heilmannii]	<u>287</u>	1e-76
<input type="checkbox"/>	tr	<u>Q5KYM1</u>	_GEOKA Urease alpha subunit (Urea amidohydrolase) (EC 3...	<u>286</u>	2e-76
<input type="checkbox"/>	tr	<u>Q8GLB4</u>	_HELHE UreB (Fragment) [Helicobacter heilmannii]	<u>286</u>	2e-76
<input type="checkbox"/>	tr	<u>Q62HS0</u>	_BURMA Urease, alpha subunit (EC 3.5.1.5) [ureC] [Burkh...	<u>285</u>	8e-76
<input type="checkbox"/>	tr	<u>Q63RL3</u>	_BURPS Urease alpha subunit (EC 3.5.1.5) [ureC] [Burkho...	<u>285</u>	8e-76
<input type="checkbox"/>	tr	<u>Q5FB23</u>	_CAMLA Urease B subunit [ureB] [Campylobacter lari]	<u>285</u>	8e-76
<input type="checkbox"/>	tr	<u>Q8GLB3</u>	_HELHE UreB (Fragment) [Helicobacter heilmannii]	<u>283</u>	2e-75
<input type="checkbox"/>	tr	<u>Q8XXT1</u>	_RALSO PROBABLE UREASE (ALPHA SUBUNIT) PROTEIN (EC 3.5....	<u>282</u>	4e-75
<input type="checkbox"/>	tr	<u>Q8GLB6</u>	_HELHE UreB (Fragment) [Helicobacter heilmannii]	<u>282</u>	5e-75
<input type="checkbox"/>	tr	<u>Q8GLB2</u>	_HELHE UreB (Fragment) [Helicobacter heilmannii]	<u>281</u>	6e-75
<input type="checkbox"/>	tr	<u>Q8YQZ0</u>	_ANASP Urease alpha subunit [alr3670] [Anabaena sp. (st...	<u>281</u>	1e-74
<input type="checkbox"/>	tr	<u>Q6I6H5</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>280</u>	2e-74
<input type="checkbox"/>	tr	<u>Q6I6I9</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>278</u>	5e-74
<input type="checkbox"/>	tr	<u>Q6I6H3</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>278</u>	5e-74
<input type="checkbox"/>	tr	<u>Q6I6I3</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>278</u>	7e-74
<input type="checkbox"/>	tr	<u>Q7V3V2</u>	_PROMM Urease alpha subunit (EC 3.5.1.5) [ureC] [Prochl...	<u>277</u>	1e-73
<input type="checkbox"/>	tr	<u>Q6I6J1</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>277</u>	1e-73
<input type="checkbox"/>	tr	<u>Q6I6I5</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>277</u>	2e-73
<input type="checkbox"/>	tr	<u>Q6I6H1</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>276</u>	2e-73
<input type="checkbox"/>	tr	<u>Q5E728</u>	_VIBF1 Urease alpha subunit (EC 3.5.1.5) [VF0673] [Vibr...	<u>276</u>	2e-73
<input type="checkbox"/>	tr	<u>Q8DMV6</u>	_SYNEL Urease alpha subunit [ureC] [Synechococcus elong...	<u>276</u>	4e-73
<input type="checkbox"/>	tr	<u>Q4ZN06</u>	_PSESY Urease (EC 3.5.1.5) [P syr_4436] [Pseudomonas syr...	<u>275</u>	8e-73
<input type="checkbox"/>	tr	<u>Q9RYJ4</u>	_DEIRA Urease, alpha subunit [DRA0318] [Deinococcus rad...	<u>274</u>	1e-72
<input type="checkbox"/>	tr	<u>Q87VP0</u>	_PSESM Urease, alpha subunit [ureC] [Pseudomonas syring...	<u>274</u>	1e-72
<input type="checkbox"/>	sp	<u>P16122</u>	URE1_PROVU Urease alpha subunit (EC 3.5.1.5) (Urea ami...	<u>274</u>	1e-72
<input type="checkbox"/>	tr	<u>Q733J6</u>	_BACC1 Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacill...	<u>273</u>	2e-72
<input type="checkbox"/>	sp	<u>P17086</u>	URE1_PROMI Urease alpha subunit (EC 3.5.1.5) (Urea ami...	<u>273</u>	3e-72
<input type="checkbox"/>	sp	<u>P73061</u>	URE1_SYNY3 Urease alpha subunit (EC 3.5.1.5) (Urea ami...	<u>272</u>	4e-72
<input type="checkbox"/>	tr	<u>Q7U3I3</u>	_SYNPX Urease alpha subunit (EC 3.5.1.5) [ureC] [Synech...	<u>272</u>	4e-72
<input type="checkbox"/>	tr	<u>Q52305</u>	_SYNP2 Urease alpha subunit [ureC] [Synechococcus sp. (...	<u>272</u>	4e-72
<input type="checkbox"/>	tr	<u>Q6I6H7</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>272</u>	5e-72
<input type="checkbox"/>	tr	<u>Q6I6I1</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>271</u>	1e-71
<input type="checkbox"/>	tr	<u>Q6I6H9</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>271</u>	1e-71
<input type="checkbox"/>	tr	<u>Q6FD83</u>	_ACIAD Urease alpha subunit (EC 3.5.1.5) [ureC] [Acinet...	<u>270</u>	1e-71
<input type="checkbox"/>	tr	<u>Q9HUU5</u>	_PSEAE Urease alpha subunit [ureC] [Pseudomonas aerugin...	<u>270</u>	3e-71
<input type="checkbox"/>	tr	<u>Q88J04</u>	_PSEPK Urease, alpha subunit [ureC] [Pseudomonas putida...	<u>270</u>	3e-71
<input type="checkbox"/>	tr	<u>Q7V1B6</u>	_PROMP Urease alpha subunit (EC 3.5.1.5) [ureC] [Prochl...	<u>268</u>	6e-71
<input type="checkbox"/>	tr	<u>Q9L644</u>	_PROMA UreC [ureC] [Prochlorococcus marinus]	<u>268</u>	6e-71
<input type="checkbox"/>	sp	<u>P77837</u>	URE1_BACSU Urease alpha subunit (EC 3.5.1.5) (Urea ami...	<u>268</u>	7e-71
<input type="checkbox"/>	tr	<u>Q6I6I8</u>	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	<u>268</u>	7e-71

Graphical overview of the alignments

[Click here](#) to resubmit your query after masking regions matching PROSITE profiles or Pfam HMMs
( [Help](#)) (use [ScanProsite](#) for more details about PROSITE matches)

Profile hits

Pfam hits

IMPDH

Submission	Matches on query sequence				Mat
	1	50	100	150	
URE2_HELFE					
Q9RGP5					
Q8KT24					
Q8KT17					
Q6UK88					
Q6UK76					
Q6UK66					
Q6UK82					
Q8KT28					
Q8KT23					
Q6UK68					
Q8KT25					
Q8GH97					
Q9X753					
Q8KT26					
Q6UK76					
Q9S6R9					
URE2_HELHE					
Q8KT16					
Q8KT27					
Q8KT31					
Q8KT32					
Q8KT22					
Q8KT21					
Q8KT18					
Q8KT29					
Q8KT19					
Q8KT13					
Q8KT20					
URE2_HELPY					
URE2_HELPJ					
Q9S0Q5					
Q8KT12					
Q84F75					
Q7X3W5					
Q6UK72					
Q64EY3					
Q9X742					
Q8KT33					
Q8RNU6					
Q8KT30					
Q8KT11					
Q9AFB1					
Q9S6R7					
Q6UK74					
Q6UK70					
Q8KT15					
URE2_HELHU					
Q9R8R3					
Q9R8R1					
Q93PJ4					
Q9ZHF1					
Q9RGP8					
Q8KT14					
Q9RGP7					
Q9RGP6					
Q9RGQ8					
Q9RGP9					
URE1_BACSB					
Q9K659					
Q8GLB5					
Q5KYH1					
Q8GLB4					
Q62HS0					
Q63RL3					
Q5FB23					
Q8GLB3					
Q8XXT1					
Q8GLB6					
Q8GLB2					
Q8YQZ0					
Q6I6H5					
Q6I6I9					
Q6I6H3					
Q6I6I3					
Q7V3V2					
Q6I6J1					
Q6I6I5					
Q6I6H1					
Q5E728					
Q8DMV6					
Q4ZN06					
Q9RYJ4					
Q87VP8					
URE1_PROVU					
Q733J6					

Alignments

sp Q08716 Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) 569
 URE2_HELFE [ureB] AA
 [Helicobacter felis] align

Score = 437 bits (1124), Expect = e-122
 Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
 Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNMLCVGPATEALAAEGL 120
 DLVLTNALIVDYT MQDGVNMLCVGPATEALAAEGL
 Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNMLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA
 Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
 Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
 IHT
 Sbjct: 247 IHT 249

tr Q9RGP5 Urease (Fragment) [ureB] [Helicobacter] 243 AA
 Q9RGP5_HELFE felis] align

Score = 437 bits (1124), Expect = e-122
 Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
 Sbjct: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNMLCVGPATEALAAEGL 120
 DLVLTNALIVDYT MQDGVNMLCVGPATEALAAEGL
 Sbjct: 61 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNMLCVGPATEALAAEGL 120

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA
 Sbjct: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
 Sbjct: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240

Query: 241 IHT 243
 IHT
 Sbjct: 241 IHT 243

tr Q8KT24 Urease UreB (Fragment) [ureB] [Helicobacter 384 AA
Q8KT24_HELFE felis] align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNTLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGVNNTLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNTLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT17 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 360 AA
Q8KT17_HELHE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNTLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGVNNTLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNTLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q6UK80 UreB (Fragment) [ureB] [Helicobacter felis] 360 AA
Q6UK80_HELFE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT M QDGVNNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDM QDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q6UK78 UreB (Fragment) [ureB] [Helicobacter felis] 360 AA
Q6UK78_HELFE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT M QDGVNNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDM QDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q6UK66 UreB (Fragment) [ureB] [Helicobacter felis] 362 AA
Q6UK66_HELFE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGVNNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA
Sbjct: 127 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 186

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q6UK82 UreB (Fragment) [ureB] [Helicobacter felis] 363 AA
Q6UK82_HELFE align

Score = 436 bits (1120), Expect = e-121
Identities = 218/243 (89%), Positives = 219/243 (89%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGVNNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA
Sbjct: 127 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 186

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E+YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EKYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT28 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 334 AA
Q8KT28_HELHE

align

Score = 435 bits (1118), Expect = e-121
Identities = 218/243 (89%), Positives = 218/243 (89%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGVNNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIG KIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGLKI HEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT23 Urease UreB (Fragment) [ureB] [Helicobacter salomonis] 359 AA
Q8KT23_9HELI

align

Score = 434 bits (1115), Expect = e-120
Identities = 217/243 (89%), Positives = 218/243 (89%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGVNNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMRLAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRNLKSMRLAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q6UK68 UreB (Fragment) [ureB] [Helicobacter] 359 AA
Q6UK68_9HELI salomonis] align

Score = 423 bits (1088), Expect = e-117
Identities = 213/243 (87%), Positives = 214/243 (87%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7 KEYVSMYGPTTGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVKNL SVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSM L AA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSM LXA A 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT25 Urease UreB (Fragment) [ureB] [Helicobacter] 386
Q8KT25_HELBI bizzoeronii] AA
align

Score = 413 bits (1062), Expect = e-114
Identities = 205/243 (84%), Positives = 215/243 (88%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNSPSS+EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGV NNLCVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDMQDGV CNL CVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLK TMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLG+L KGNVSYEPSL DQ+EAGAIGFKIHEDWGSTPAAI+HCLNVAD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSYEPSLVDQLEAGAIGFKIHEDWGSTPAAIYHCLNVADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8GH97 Urease B [*Helicobacter bizzozeronii*] 569 AA
Q8GH97_HELBI align

Score = 413 bits (1062), Expect = e-114
Identities = 205/243 (84%), Positives = 215/243 (88%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QINSFSS+EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
DLVLTNALIVDYT MQDGV NNL CVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDMQDGV CNL CVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKTMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLG+L KGNVSYEPSL DQ+EAGAIGFKIHEDWGSTPAAT+HCLNVAD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSYEPSLVDQLEAGAIGFKIHEDWGSTPAAIYHCLNVADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q9X753 Urease (Fragment) [ureB] [*Helicobacter*] 231 AA
Q9X753_9HELI salomonis align

Score = 409 bits (1051), Expect = e-113
Identities = 205/231 (88%), Positives = 206/231 (88%)

Query: 9 PTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNAL 68
PTTGDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNAL
Sbjct: 1 PTTGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNAL 60

Query: 69 IVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGLIVTAGGID 128
IVDYTX MQDGVNNL CVGPATEALAAEGLIVTAGGID
Sbjct: 61 IVDYTGIIYKADIGIKDKGIAGIGKAGNKDMQDGVNNL CVGPATEALAAEGLIVTAGGID 120

Query: 129 THIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAMNLG 188
THIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMRLRAAEYAMNLG
Sbjct: 121 THIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLRAAEYAMNLG 180

Query: 189 FLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
FLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV
Sbjct: 181 FLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 231

tr Q8KT26 Urease UreB (Fragment) [ureB] [*Helicobacter*] 377
Q8KT26_HELBI bizzozeronii align

Score = 409 bits (1051), Expect = e-113

Identities = 201/243 (82%), Positives = 213/243 (86%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
          KEYVSMYGPTTGD+VRLGDTDLIL VEHDCTTYGEEIKFGGGKTIRDGM+QTNSSPS+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILXVEHDCTTYGEEIKFGGGKTIRDGMAQTNSSPSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
          DLVLTNALIVDYT                                MQDGV NNL CVGPATEALAAEGL
Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDMQDGVNNL CVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFI SPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITT MIGGGTGPADGTNATTITPGRWNLKTM LRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG+L KGNVSYEPSL DQ+EAGAIGFKIHEDWGSTPAAIHHCLNVAB+YBVQVA
Sbjct: 187 XEYAMNLGYLGKGNVSYEPSLVDQLEAGAIGFKIHEDWGSTPAAIHHCLNVABKYBVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249

```

```

tr Q6UK76      UreB (Fragment) [ureB] [Helicobacter pylori]      360
Q6UK76_HELPY (Campylobacter pylori)]                          AA
                                                                align

```

Score = 408 bits (1048), Expect = e-113
 Identities = 202/243 (83%), Positives = 211/243 (86%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
          KEYVSMYGPTTGD+VRLGDTDLI EVEND T YGEE+KFGGGKT+K+GMSQ+N+PS EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
          DL++TNALIVDYT                                MQDGV NNL CVGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVNNL CVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSM LRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVAD+YDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADKYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249

```

```

tr Q9S6R9      Urease (Fragment) [ureB] [Helicobacter      231 AA
Q9S6R9_9HELI  salomonis]                                align

```

Score = 403 bits (1035), Expect = e-111

Identities = 202/229 (88%), Positives = 204/229 (88%)

```

Query: 11  TGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIV 70
          +GDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIV
Sbjct: 3   SGRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIV 62

Query: 71  DYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAGGIDTH 130
          DYT                                MQDGVDDNNLCVGPATEALAAEGLIVTAGGIDTH
Sbjct: 63  DYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDDNNLCVGPATEALAAEGLIVTAGGIDTH 122

Query: 131  IHFISPPQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEEYAMNLGFL 190
          IHFISPPQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMRLAAEEYAMNLGFL
Sbjct: 123  IHFISPPQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMRLAAEEYAMNLGFL 182

Query: 191  AKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
          AKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV
Sbjct: 183  AKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 231

```

sp P42823 Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) 568
 URE2_HELHE [ureB] AA
 [Helicobacter heilmannii] align

Score = 400 bits (1028), Expect = e-110
 Identities = 197/243 (81%), Positives = 211/243 (86%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
          KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGL 120
          DLV+TNALIVDYT                                +QDGV N LCVGPATEALAAEGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDLQDGVNRLCVGPATEALAAEGL 126

Query: 121  IVTAGGIDTHIHFISPPQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
          IVTAGGIDTHIHFISPPQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127  IVTAGGIDTHIHFISPPQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG+L KGNVS+EP+L DQ+EAGAIGFKIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187  EEYAMNLGYLGKGNVSFEPALIDQLEAGAIGFKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241  IHT 243
          IHT
Sbjct: 247  IHT 249

```

tr Q8KT16 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 358 AA
 Q8KT16_HELHE align

Score = 400 bits (1028), Expect = e-110
 Identities = 197/243 (81%), Positives = 211/243 (86%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60

```

Sbjct: 7 KEYVSMYGPTTGDL+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPS+EL 66
Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGVNLCVGPATEALAAEGL 120
DLV+TNALIVDYT +QDGV N LCVGPATEALAAEGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKNGKIHGIGKAGNKDLQDGVNLCVGPATEALAAEGL 126
Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWN LKEMLRAS 186
Query: 181 E EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E EYAMNLG++L KGNVS+EP+L DQ+EAGAIGFKIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187 E EYAMNLGYLGKGNVSFEPALIDQIEAGAIGFKIHEDWGSTPSAINHALNIADKYDVQVA 246
Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT27 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 357 AA
Q8KT27_HELHE align

Score = 400 bits (1027), Expect = e-110
Identities = 196/243 (80%), Positives = 209/243 (85%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDL+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPS+EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPSHEL 66
Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGVNLCVGPATEALAAEGL 120
DLV+TNALIVDYT +QDGV N LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDKGIHGIGKAGNKDIQDGVNLCVGPATEALAGEGL 126
Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASG+TTM+GGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMLGGGTGPADGTNATTITPGRWN LKEMLRAS 186
Query: 181 E EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E EYAMNLG++ KGNVSVEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 E EYAMNLGYMGKGNVSVEPSLVEQLEAGAIGFKIHEDWGSTPSAIH HALKIADEYDVQVA 246
Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT31 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 355 AA
Q8KT31_HELHE align

Score = 398 bits (1023), Expect = e-110
Identities = 196/243 (80%), Positives = 210/243 (85%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPS+EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMQTNSSPSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGV DNNLCVGPATEALAAEGL 120
DLV+TNALIVDYT +QDGV N LCVGPATEALAAEGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKNGKIHGIGKAGNKDLQDGV CNRLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASG+TTMIGGGTGPADGTNA TTTPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMIGGGTGPADGTNAXTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLG+L KGNVS+EP+L DQ+EAGAIGFKIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSFEPALIDQLEAGAIGFKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT32 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 358 AA
Q8KT32_HELHE

align

Score = 398 bits (1022), Expect = e-110
Identities = 195/243 (80%), Positives = 208/243 (85%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPS+EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMQTNSSPSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGV DNNLCVGPATEALAAEGL 120
DLV+TNALIVDYT +QDGV N LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGV CNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT22 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 356 AA
Q8KT22_HELHE

align

Score = 398 bits (1022), Expect = e-110
Identities = 195/243 (80%), Positives = 208/243 (85%)

Query: 1 KEYVSMYGPTTGDRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPY+EL
Sbjct: 7 KEYVSMYGPTTGDVKRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMQTNSSPSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
DLV+TNALIVDYT +QDGV N LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVNLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFISSPQQIPTAFASG+TEM+GGGTGPADGTNATTITPG NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLIEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT21 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 358 AA
Q8KT21_HELHE

align

Score = 398 bits (1022), Expect = e-110
Identities = 195/243 (80%), Positives = 208/243 (85%)

Query: 1 KEYVSMYGPTTGDRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPY+EL
Sbjct: 7 KEYVSMYGPTTGDVKRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMQTNSSPSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
DLV+TNALIVDYT +QDGV N LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVNLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFISSPQQIPTAFASG+TEM+GGGTGPADGTNATTITPG NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLIEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT18 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 357 AA
Q8KT18_HELHE

align

Score = 398 bits (1022), Expect = e-110

Identities = 195/243 (80%), Positives = 208/243 (85%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
          KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
          DLV+TNALIVDYT                      +QDGV N LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASG+TMM+GGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRHWNLEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHLKIADEYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249
```

tr Q8KT29 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 379 AA
Q8KT29_HELHE

align

Score = 394 bits (1013), Expect = e-109
Identities = 195/243 (80%), Positives = 208/243 (85%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
          KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
          DLV+TNALIVDYT                      QDGV N LCVGPATEALAAEGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKNGKIHGIGKAGNKDXQDGVNRLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASG+TMMIGGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRHWNLEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG+L KGNVS+EP+ DQ+EAGAIG KIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSFEPAXIDQLEAGAIGXKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249
```

tr Q8KT19 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 357 AA
Q8KT19_HELHE

align

Score = 392 bits (1006), Expect = e-108
Identities = 193/243 (79%), Positives = 207/243 (84%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
          KEYVSMYGPTTGD+VRLGDTDLILEVE DCTTYGEEIKFGGGKTIRDGM QTNPSSE+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVECDCTTYGEEIKFGGGKTIRDGMGQTNPSSEHSL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGV DNNLCVGPATEALAAEGL 120
          DLV+TNALIVDYT +QDGV + LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVCDRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249
```

tr Q8KT13 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 357 AA
Q8KT13_HELHE

align

Score = 392 bits (1006), Expect = e-108
Identities = 193/243 (79%), Positives = 207/243 (84%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
          KEYVSMYGPTTGD+VRLGDTDLILEVE DCTTYGEEIKFGGGKTIRDGM QTNPSSE+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVECDCTTYGEEIKFGGGKTIRDGMGQTNPSSEHSL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGV DNNLCVGPATEALAAEGL 120
          DLV+TNALIVDYT +QDGV + LCVGPATEALA EGL
Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVCDRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249
```

tr Q8KT20 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 356 AA
Q8KT20_HELHE

align

Score = 389 bits (1000), Expect = e-107
Identities = 192/243 (79%), Positives = 206/243 (84%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYEL 60
          KEYVSMYGPTTGD+VRLGDTDLILEVE DCTTYGEEIKFGGGKTIRDGM QTNSSPS+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVECDCTTYGEEIKFGGGKTIRDGMGQTNSSPSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
          DLV+TNALIVDYT . +QDGV + LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVCDRLCVGPATEALAGEGL 126

Query: 121  IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
          IVTAGGIDTHIHFI SPQQIPTAFASG+T M+GGGTGPADGTNATTITPG NLK MLRA+
Sbjct: 127  IVTAGGIDTHIHFI SPQQIPTAFASGITPMLGGGTGPADGTNATTITPGHWN LKEMLRAS 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187  EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241  IHT 243
          IHT
Sbjct: 247  IHT 249

```

sp P69996 Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) 569
 URE2_HELPY [ureB] AA
 [Helicobacter pylori (Campylobacter pylori)] align

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYEL 60
          KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
          DL++TNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121  IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
          IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127  IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKWMLRAA 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187  EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241  IHT 243
          IHT
Sbjct: 247  IHT 249

```

sp P69997 Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) 569
 URE2_HELPJ [ureB] AA
 [Helicobacter pylori J99 (Campylobacter pylori J99)] align

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
          KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNMLCVGPATEALAAEGL 120
          DL++TNALIVDYT                      MQDGV NML VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNLKWLMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249
```

tr Q9S0Q5 Urease B [ureB] [Helicobacter pylori (Campylobacter 569
Q9S0Q5_HELPY pylori)] AA
[align](#)

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
          KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNMLCVGPATEALAAEGL 120
          DL++TNALIVDYT                      MQDGV NML VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNLKWLMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249
```

tr Q8KT12 Urease UreB (Fragment) [ureB] [Helicobacter pylori SS1] 378 AA
Q8KT12_HELPY
[align](#)

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTI RDGMSQTNPSSEYEL 60
KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGV DNNLCVGPATEALAAEGL 120
DL++TNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q84F75 Urease beta (EC 3.5.1.5) [ureB] [Helicobacter pylori] 569
Q84F75_HELPY (Campylobacter pylori)] AA
align

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTI RDGMSQTNPSSEYEL 60
KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGV DNNLCVGPATEALAAEGL 120
DL++TNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q7X3W5 Urease B [ureB] [Helicobacter pylori (Campylobacter] 569
Q7X3W5_HELPY pylori)] AA
align

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
          KEYVSMYGPTTGD+VRLGDTDLI  EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVGPATEALAAEGL 120
          DL++TNALIVDYT                      MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121  IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127  IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187  EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241  IHT 243
          IHT
Sbjct: 247  IHT 249
```

tr Q6UK72 UreB (Fragment) [ureB] [Helicobacter pylori] 370
Q6UK72_HELPY (Campylobacter pylori)] AA
align

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

```
Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
          KEYVSMYGPTTGD+VRLGDTDLI  EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVGPATEALAAEGL 120
          DL++TNALIVDYT                      MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121  IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127  IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187  EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241  IHT 243
          IHT
Sbjct: 247  IHT 249
```

tr Q64EY3 UreB [ureB] [Helicobacter pylori (Campylobacter pylori)] 567 AA
Q64EY3_HELPY
align

Score = 378 bits (970), Expect = e-104
Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 5 KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 64

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
DL++TNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 65 DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDMQDGVKNLSVGPATEALAGEGL 124

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 125 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 184

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEE+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 185 EEYSMNLGFLAKGNTSNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 244

Query: 241 IHT 243
IHT
Sbjct: 245 IHT 247

tr Q9X742 Urease (Fragment) [ureB] [Helicobacter bizzozeronii] 231 AA
Q9X742_HELBI

align

Score = 377 bits (968), Expect = e-103
Identities = 188/224 (83%), Positives = 196/224 (86%)

Query: 17 LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYELDLVLTNALIVDYTXXX 76
LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNPSSE+ELDLVLTNALIVDYT
Sbjct: 8 LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNPSSEHLDLVLTNALIVDYTGIIY 67

Query: 77 XXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGLIVTAGGIDTHIHFISSP 136
MQDGV NNLVGPATEALAAEGLIVTAGGIDTHIHFISSP
Sbjct: 68 KADIGIKNGKIHGIGKAGNKDMQDGVNLCVGPATEALAAEGLIVTAGGIDTHIHFISSP 127

Query: 137 QQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVS 196
QQIPTAFASG+TMMIGGGTGPADGTNATTITPGR NLK+MLRA+EEYAMNLG+L KGNVS
Sbjct: 128 QQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLTMLRASEEYAMNLGYLGKGNVS 187

Query: 197 YEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
YEPSL DQ+EAGAIGFKIHEDWGSTPAAIHHCLNVAD+YDVQVA
Sbjct: 188 YEPSLVDQLEAGAIGFKIHEDWGSTPAAIHHCLNVADKYDVQVA 231

tr Q8KT33 Urease UreB (Fragment) [ureB] [Helicobacter pylori] 377
Q8KT33_HELPY 26695]

AA
align

Score = 377 bits (968), Expect = e-103
Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLLCVGPATEALAAEGL 120
DL++TNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLVDQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8RNU6 Urease B subunit [ureB] [Helicobacter pylori] 559
Q8RNU6_HELPY (Campylobacter pylori)] AA
align

Score = 376 bits (966), Expect = e-103
Identities = 188/243 (77%), Positives = 203/243 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEY SMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
Sbjct: 7 KEYASMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLLCVGPATEALAAEGL 120
DL++TNALIVDYT MQDGV NNL VGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q8KT30 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 222 AA
Q8KT30_HELHE
align

Score = 376 bits (966), Expect = e-103
Identities = 191/216 (88%), Positives = 191/216 (88%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL
 Sbjct: 7 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
 DLVLTNALIVDYT MQDGVNNLCVGPATEALAAEGL
 Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
 Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHE 216
 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIG KHE
 Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGLKIHE 222

tr Q8KT11 Urease UreB (Fragment) [ureB] [Helicobacter sp. TD1] 387 AA
 Q8KT11_9HELI

align

Score = 376 bits (965), Expect = e-103
 Identities = 189/243 (77%), Positives = 203/243 (82%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
 KEYVSMYGPTTGDRVRLGDTDLILEVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL
 Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
 DLVLTNALIVDYT MQDGV NNL VGPATEALA EGL
 Sbjct: 67 DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNLNVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 IVTAGGIDTHI FISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLE MLRAA
 Sbjct: 127 IVTAGGIDTHIXFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 EEYAMNLGFL KGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
 Sbjct: 187 EEYAMNLGFLGKNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
 IHT
 Sbjct: 247 IHT 249

tr Q9AFB1 Urease B [Helicobacter pylori (Campylobacter pylori)] 569 AA
 Q9AFB1_HELPY

align

Score = 375 bits (964), Expect = e-103
 Identities = 188/243 (77%), Positives = 203/243 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
 KEYVSMYGPTTGDRVRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL

Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVCGPATEALAAEGL 120
DL++TNALIVDYT MQDGV NNL VGPATEALA EGL

Sbjct: 67 DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDMQDGVKNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NIK MLRAA

Sbjct: 127 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKLWMLRAA 186

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
EEY+MNL FLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA

Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
IHT

Sbjct: 247 IHT 249

tr Q9S6R7 Urease (Fragment) [ureB] [Helicobacter bizzozeronii] 230 AA
Q9S6R7_HELBI align

Score = 373 bits (958), Expect = e-102
Identities = 186/223 (83%), Positives = 195/223 (87%)

Query: 17 LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLTNALIVDYTXXX 76
LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNSSPS+ELDLVLTNALIVDYT

Sbjct: 8 LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSSSHELDDLVLTNALIVDYTGIIY 67

Query: 77 XXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVCGPATEALAAEGLIVTAGGIDTHIHFISSP 136
MQDGV NNLVCGPATEALAAEGLIVTAGGIDTHIHFISSP

Sbjct: 68 KADIGIKNGKIHGIGKAGNKDMQDGVNNLVCGPATEALAAEGLIVTAGGIDTHIHFISSP 127

Query: 137 QQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVS 196
QQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NIK+MLRA+EEYAMNLG+L KGNVS

Sbjct: 128 QQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLTMLRASEEYAMNLGYLGKGNVS 187

Query: 197 YEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
YEPSL DQ+EAGAIGFKIHEDWGSTPAAI+HCLNVADEYDVQV

Sbjct: 188 YEPSLVDQIEAGAIGFKIHEDWGSTPAAIYHCLNVADEYDVQV 230

tr Q6UK74 UreB (Fragment) [ureB] [Helicobacter pylori] 359
Q6UK74_HELPY (Campylobacter pylori)] AA
align

Score = 372 bits (955), Expect = e-102
Identities = 186/243 (76%), Positives = 201/243 (82%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
KEY SMYGPTTGDRVRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS EL

Sbjct: 7 KEYASMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVCGPATEALAAEGL 120

```

          DL++TINALIVDYT                      MQDGV>NNL VGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDMQDGVKNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKFMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MN GFLAKGN S + SL DQIEAGAIG KIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNFGFLAKGNXSNLASLADQIEAGAIGXKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249

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tr Q6UK70          UreB (Fragment) [ureB] [Helicobacter pylori]          374
   Q6UK70_HELPY  (Campylobacter pylori)]                               AA
                                                                align

```

Score = 372 bits (955), Expect = e-102
 Identities = 186/243 (76%), Positives = 201/243 (82%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
          KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTIREGMSQSNNPSKEEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
          DL++TINALIVDYT                      QDGV>NNL VGPATEALA EGL
Sbjct: 67 DLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKDTQDGVKNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
          IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLNKFMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
          EEY+MN GFLAKGN S + SL DQIEAGAIG KIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNFGFLAKGNASNDASLADQIEAGAIGLKIHDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
          IHT
Sbjct: 247 IHT 249

```

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tr Q8KT15          Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 222 AA
   Q8KT15_HELHE                                         align

```

Score = 346 bits (888), Expect = 2e-94
 Identities = 172/216 (79%), Positives = 183/216 (84%)

```

Query: 1  KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
          KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7  KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMQTNSPSSHEL 66

```

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
 DLV+TNALIVDYT +QDGV N LCVGPATEALA EGL
 Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVNRL CVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 IVTAGGIDTHIHFI SPQQIPTAFASG+TTM+GGGTGPADGTNATTITPGR NIK MLRA+
 Sbjct: 127 IVTAGGIDTHIHFI SPQQIPTAFASGITTMLGGGTGPADGTNATTITPGRWNLEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHE 216
 EEYAMNLG++ KGNVSYEPSL +Q+EAGAIG KHE
 Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGLKIHE 222

sp P50045 Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) 308
 URE2_HELMU (Fragment) AA
 [ureB] [Helicobacter mustelae] align

Score = 323 bits (829), Expect = 1e-87
 Identities = 163/243 (67%), Positives = 185/243 (76%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN SPSSYEL 60
 KEYVSMYGPTTGDRVRLGDT+LI E+E D T YGEEIKFGGGKTIRDGMSQ+ SP EL
 Sbjct: 7 KEYVSMYGPTTGDKVRLGDTLIEAEIEKDYTVYGEEIKFGGGKTIRDGMSQSVSPDVNEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
 D V+TNA+I+DYT QDGV +L VG +TEA+A EGL
 Sbjct: 67 DAVITNAMIIDYTGIIYKADIGIKDGKIAGIGKAGNRDTQDGVGMDLVVGASTEALAGEGL 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 IVTAGGIDTHIHFI SP QIPFA SGVTTMIGGGTGEA GT ATT+PG N+K M+RAA
 Sbjct: 127 IVTAGGIDTHIHFI SPQIPTALYSGVTTMIGGGTGPAAAGTFATTISPGEWNIKQMIRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 EEY MNLGF KGN S +L DQI+AGA+GFK+HED GSTFA I+H L++A++YDVQNA
 Sbjct: 187 EEYTMNLGFFGKGNTSNVKALEDQIKAGALGFKVHEDCGSTPAVINHSLDIAEKYDVQVA 246

Query: 241 IHT 243
 IHT
 Sbjct: 247 IHT 249

tr Q9R8R3 Urease beta subunit (Fragment) [ureB] [Helicobacter 315
 Q9R8R3_HELHP hepaticus] AA
align

Score = 319 bits (817), Expect = 4e-86
 Identities = 159/243 (65%), Positives = 181/243 (74%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN SPSSYEL 60
 K+Y SMYGPTTGDRVRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ S + EL
 Sbjct: 7 KQYASMYGPTTGDKVRLGDTNLF AEIEKDYTL YGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNL CVGPATEALAAEGL 120
 D V+TNA+I+DYT QDGV+ + VG ATE +A EG
 Sbjct: 67 DAVITNAMIIDYTGIIYKADIGIKGGKIVGIGKAGNPDTQDGVNEAMVVGAAATEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFI SPQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
I+TAGGIDTHIHFI SP QIPTA SGVTTMIGGGTGP A GTNATT TPG+ N+ MLRAA
Sbjct: 127 IITAGGIDTHIHFI SPQIPTALYSGVTTMIGGGTGP AAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 E EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E YAMNLGF KGN S E L +QT+AGA+G K+HEDWGSTPAAL+H LNVA +YDVQVA
Sbjct: 187 ESYAMNLGFFFGKGNSSNEEGLEE QIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVA 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q9R8R1 Urease beta subunit (Fragment) [ureB] [Helicobacter 315
Q9R8R1_HELHP hepaticus] AA
align

Score = 317 bits (812), Expect = 1e-85
Identities = 158/243 (65%), Positives = 180/243 (74%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
K+Y SMYGPTTGD+VRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ S + EL
Sbjct: 7 KQYASMYGPTTGDKVRLGDTNLFAEIEKD YTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61 DLVLTNALIVDY TXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGV DNNLCVGPATEALAAEGL 120
D V+TNA+I+DYT QDGV+ + VG ATE +A EG
Sbjct: 67 DAVITNAMIIDY TGIYKADIGIKGGKIVGIGKAGNPDTQDGVNEAMVVGAA TEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFI SPQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM LRAA 180
I+TAGGIDTHIHFI SP QIPTA SGVTTMIGGGTGP A GTNATT TPG+ N+ MLRAA
Sbjct: 127 IITAGGIDTHIHFI SPQIPTALYSGVTTMIGGGTGP AAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 E EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E YAMNLGF KGN S E L +QT+AGA+G K+HEDWGSTPAAL+H LNVA +YDVQV
Sbjct: 187 ESYAMNLGFFFGKGNSSNEEGLXE QIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVV 246

Query: 241 IHT 243
IHT
Sbjct: 247 IHT 249

tr Q93PJ4 Urease beta subunit UreB (EC 3.5.1.5) [ureB] 569
Q93PJ4_HELHP [Helicobacter AA
hepaticus] align

Score = 317 bits (811), Expect = 2e-85
Identities = 158/243 (65%), Positives = 180/243 (74%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
K+Y SMYGPTTGD+VRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ S + EL
Sbjct: 7 KQYASMYGPTTGDKVRLGDTNLFAEIEKD YTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61 DLVLTNALIVDY TXXXXXXXXXXXXXXXXXXXXXXXXXXXXM QDGV DNNLCVGPATEALAAEGL 120
D V+TNA+I+DYT QD V+ + VG ATE +A EG

Sbjct: 67 DAVITNAMIIDYTGIIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAAATEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
I+TAGGIDTHIHFI SP QIPTA SGVTTMIGGGTGP GINATT TFG+ N+ MLRAA

Sbjct: 127 IITAGGIDTHIHFI SPQTALYSGVTTMIGGGTGPAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E YAMNLGF KGN S E L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA

Sbjct: 187 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVA 246

Query: 241 IHT 243
IHT

Sbjct: 247 IHT 249

tr Q9ZHF1 Urease beta subunit (Fragment) [ureB] [Helicobacter 315
Q9ZHF1_HELHP hepaticus] AA
align

Score = 317 bits (811), Expect = 2e-85
Identities = 158/243 (65%), Positives = 180/243 (74%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
K+Y SMYGPTTGDRVRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ S + EL

Sbjct: 7 KQYASMYGPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGL 120
D V+TNA+I+DYT QD V+ + VG ATE +A EG

Sbjct: 67 DAVITNAMIIDYTGIIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAAATEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
I+TAGGIDTHIHFI SP QIPTA SGVTTMIGGGTGP GINATT TFG+ N+ MLRAA

Sbjct: 127 IITAGGIDTHIHFI SPQTALYSGVTTMIGGGTGPAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
E YAMNLGF KGN S E L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA

Sbjct: 187 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVA 246

Query: 241 IHT 243
IHT

Sbjct: 247 IHT 249

tr Q9RGP8 Urease (Fragment) [ureB] [Flexispira 243 AA
Q9RGP8_9HELI rappini] align

Score = 312 bits (800), Expect = 3e-84
Identities = 155/243 (63%), Positives = 180/243 (73%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
KEYVSMYGPTTGDRVRLGDT+L E+E D YGEEIKFGGGKTIRDGM+Q+ S S EL

Sbjct: 1 KEYVSMYGPTTGDKIRLGDTLFAEIEKDYAIYGEEIKFGGGKTIRDGMAQSVSDSENEL 60

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGL 120
D V+TNA+I+DYT QDGV + L VG TE +A EGL

Sbjct: 61 DSVITNAVIIDYTGIIYKADIGIKNGKIFGIGKAGNKDTQDGVCDKLIVGTNTEVIAGEGL 120

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
 IVTAGGIDTHIH+ISP QIPTA SGVTTMIGGGTGPAT GT+ATT TPG +++ M+RA

Sbjct: 121 IVTAGGIDTHIHFIISPQQIPTALYSGVTTMIGGGTGPAGTSATTCTPGSWHMREMIRAT 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 + YAMN GF KGN S E +L QIE+GA+G K+HEDWGSTPAAL+H LN+AD+YDVQVA

Sbjct: 181 QHYAMNFGFFGKGNSSNENALSKQIESGALGLKVHEDWGSTPAAINHALNIADKYDVQVA 240

Query: 241 IHT 243
 IHT

Sbjct: 241 IHT 243

tr Q8KT14 Urease UreB (Fragment) [ureB] [Helicobacter heilmannii] 202 AA
Q8KT14_HELHE align

Score = 311 bits (798), Expect = 6e-84
 Identities = 155/196 (79%), Positives = 164/196 (83%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
 KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL

Sbjct: 7 KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGTNSPSSHEL 66

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVCGPATEALAAEGL 120
 DLV+TNALIVDYT +QDGV N LCVGPATEALA EGL

Sbjct: 67 DLVITNALIVDYTGIIYKADIGIKDGKIHGIGKAGNKDIQDGVNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
 IVTAGGIDTHIHFIISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPGR NLK MLRA+

Sbjct: 127 IVTAGGIDTHIHFIISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVS 196
 E YAMNLG++ KGNVS

Sbjct: 187 EXYAMNLGYMGKGNVS 202

tr Q9RGP7 Urease (Fragment) [ureB] [Helicobacter 243 AA
Q9RGP7_HELHP hepaticus align

Score = 311 bits (796), Expect = 1e-83
 Identities = 156/243 (64%), Positives = 177/243 (72%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
 KEY SMY PTTGD+VRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ + EL

Sbjct: 1 KEYASMYAPTTGDKVRLGDTNLFAEIEKDYTLTYGEEIKFGGGKTIRDGMAQSAXTYTNEL 60

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLVCGPATEALAAEGL 120
 D V+TNA+I+DYT QD V+ + VG ATE +A EG

Sbjct: 61 DAVITNAMIIDYTGIIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAAATEVIAGEGX 120

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
 I+TAGGIDTHIHFIISP QIPTA SGVTTMIGG TGPA GTNATT TPG+ N+ MLRAA

Sbjct: 121 IITAGGIDTHIHFIPTAXYSGVTTMIGGXTGPAAGTNATTCTPGKWNMHQMLRAA 180

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCNLVADEYDVQVA 240
 E YAMNLGF KGN S E L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA

Sbjct: 181 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAXKYDVQVA 240

Query: 241 IHT 243
 IHT

Sbjct: 241 IHT 243

tr Q9RGP6 **Urease (Fragment) [ureB] [Helicobacter** 243 AA
 Q9RGP6_HELHP **hepaticus]** align

Score = 310 bits (793), Expect = 2e-83
 Identities = 155/243 (63%), Positives = 176/243 (71%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
 KEY SMYGPTTGDRVRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ + EL

Sbjct: 1 KEYASMYGPTTGDKVRLGDTNLF AEIEKDYTL YGEEIKFGGGKTIRDGMAQSAXTYTNEL 60

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGV DNNLCVGPATEALAAEGL 120
 D V+TNA+I+DYT QD V+ + VG ATE +A EG

Sbjct: 61 DAVITNAMIIDYTG IYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVGAATEVIAGEGQ 120

Query: 121 IVTAGGIDTHIHFIPTAFASGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 180
 I+TAGGIDTHIHFIPTAFASGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA

Sbjct: 121 IITAGGIDTHIHFIPTALYSGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 180

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCNLVADEYDVQVA 240
 E YAMNLGF KGN S E L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA

Sbjct: 181 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQCA 240

Query: 241 IHT 243
 HT

Sbjct: 241 SHT 243

tr Q9RGQ0 **Urease (Fragment) [ureB] [Flexispira** 243 AA
 Q9RGQ0_9HELI **rappini]** align

Score = 308 bits (790), Expect = 5e-83
 Identities = 153/243 (62%), Positives = 178/243 (72%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
 KEY SMYGPTTGDRVRLGDT+L E+E D YGEEIKFGGGKTIRDGM+Q+ S S EL

Sbjct: 1 KEYXSMYGPTTGDKIRLGDTLFAEIEKDYAIY GEEIKFGGGKTIRDGMAQSVSDSENEL 60

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGV DNNLCVGPATEALAAEGL 120
 D V+TNA+I+DYT QDGV + L VG TE +A EGL

Sbjct: 61 DSVITNAVIIDYTG IYKADIGIKNGKIFGIGKAGNKDTQDGVCDKLIVGTNTEVIAGEGL 120

Query: 121 IVTAGGIDTHIHFIPTAFASGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 180
 VTAGGIDTHIH+ISP QIPTA SGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA

Sbjct: 121 XVTAGGIDTHIHFIPTALYSGVTTMIGGGTGPAAGTSATTCTPGSWHMRMIRAT 180

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 + YAMN GF KGN S E +L QIE+GA+G K+HEDWGSTPAAI+H LN+AD+YDVQVA
 Sbjct: 181 QHYAMNFGFFGKGNSSNENALSKQIESGALGLKVHEDWGSTPAAINHALNIADKYDVQVA 240

Query: 241 IHT 243
 IHT
 Sbjct: 241 IHT 243

tr Q9RGP9 Urease (Fragment) [ureB] [Flexispira 243 AA
 Q9RGP9_9HELI rappini] align

Score = 308 bits (790), Expect = 5e-83
 Identities = 153/243 (62%), Positives = 179/243 (72%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
 KEYVSMYGPTTGDT++RLGDT+L E+E D YGEE+KFGGGKTIRDGM+Q+ S S EL
 Sbjct: 1 KEYVSMYGPTTGDKIRLGDTLFAEIEKDYAIYGEVVKFGGGKTIRDGMAQSVSDSENEL 60

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMVDGVDNNLCVGPATEALAAEGL 120
 D V+TNA+I+DYT QDGV + L VG TE +A EGL
 Sbjct: 61 DSVITNAVIIDYTGIIYKADIGIKNGKIFGIGKAGNKDTQDGVCDKLIVGTNTEVIAGEGL 120

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
 IVTAGGIDTHIR+ISP QIPTA SGVTTMIGGGTGPRA GT+ATT TPG +++ M+RA
 Sbjct: 121 IVTAGGIDTHIHYISPTQIPTALYSGVTTMIGGGTGPAAAGTSATTCTPGSWHMREMIRAT 180

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 + YAMN GF KGN S E +L QIE+GA+G K+HEDWGSTPAAI+H L+ AD+YDVQVA
 Sbjct: 181 QHYAMNFGFFGKGNSSNENALSKQIESGALGLKVHEDWGSTPAAINHALSXADKYDVQVA 240

Query: 241 IHT 243
 IHT
 Sbjct: 241 IHT 243

sp Q07397 Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) 569
 URE1_BACSB [ureC] AA
 [Bacillus sp. (strain TB-90)] align

Score = 295 bits (756), Expect = 4e-79
 Identities = 151/244 (61%), Positives = 176/244 (71%), Gaps = 3/244 (1%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEY- 59
 K+Y M+GPT GD +RL D++L +E+E D TTYG+E+KFGGGK IRDGM Q +S E
 Sbjct: 8 KQYADMFGPTVGDAILRADSELFIEIKDYTTYGDEVKFGGGKVIRDMGMQHPLATSDEC 67

Query: 60 LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMVDGVDNNLCVGPATEALAAEG 119
 +DLVLTNAL+IVDYT + DGV D + +G ATE +AAEG
 Sbjct: 68 VDLVLTNALIVDYTGIIYKADIGIKDGMISIGKAGNPLIMDGV D--MVIGAATEVIAAEG 125

Query: 120 LIVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLA 179
 +IVTAGGID THIEI PQQI TA ASGVTTMIGGGTGPRA GTNATT TPG N+ ML+A
 Sbjct: 126 MIVTAGGIDAHIHFISSPQQIETALASGVTTMIGGGTGPATGTNATTCTPGPWNHRMLQA 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCNLNVADEYDVQV 239
 AEE+ +NLGFL KGN S E L++QIEAGA+G K+HEDWGST AAI CL VAD YDVQV
 Sbjct: 186 AEEFPINLGFLGKGNCSDEAPLKEQIEAGAVGLKLHEDWGSTAAAIIDTCLKVADRYDVQV 245

Query: 240 AIHT 243
 AIHT
 Sbjct: 246 AIHT 249

tr Q9KG59 Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacillus 571
 Q9KG59_BACHD halodurans] AA
align

Score = 289 bits (740), Expect = 3e-77
 Identities = 144/244 (59%), Positives = 176/244 (72%), Gaps = 2/244 (0%)

Query: 2 EYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSE-- 59
 ++ S+YGPT GD+VRL DTDL+LE+E D T YG+E KFGGSK +PDGM Q+ + E
 Sbjct: 7 QHASLYGPTVGDKVRLADTDLLLEIEKDYTVYGDECKFGGGKVLRDGMGQSAVYTRDEGV 66

Query: 60 LDIVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEG 119
 LEL++TNA I+DYT + DGV++++ VG +TEA+A EG
 Sbjct: 67 LDLIITNATIIDYTGIVKADIGIKDGHIVGIGKGGNPDIMDGVESHMIVGASTEAIAGEG 126

Query: 120 LIVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLEA 179
 LIVTAGGID HIFISPPQI A ASG+TMM+GGGTGPA GT ATT TPG+ N++ ML A
 Sbjct: 127 LIVTAGGIDAHIFISPPQIDVAIASGITTMIGGGTGPATGKATTCTPGKWNIERMLEA 186

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCNLNVADEYDVQV 239
 A+ + +NLGFL KGN S LR+QIEAGAIG K+HEDWG+TPAAI CL+VAD DVQV
 Sbjct: 187 ADAFPVNLGFLGKGNASTPAPLREQIEAGAIGLKLHEDWGTTTAAIRTCLSVADRMVDVQV 246

Query: 240 AIHT 243
 AIHT
 Sbjct: 247 AIHT 250

tr Q8GLB5 UreB (Fragment) [Helicobacter heilmannii] 181 AA
 Q8GLB5_HELHE align

Score = 287 bits (735), Expect = 1e-76
 Identities = 145/181 (80%), Positives = 151/181 (83%)

Query: 16 RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYELDLVLTNALIVDYTX 75
 RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNPSSE+ELDLV+TNALIVDYT
 Sbjct: 1 RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNPSSEHDLVITNALIVDYTG 60

Query: 76 XXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGLIVTAGGIDTHIHFI 135
 +QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFI
 Sbjct: 61 YKADIGIKNGKIHGIGKAGNKDLQDGVNRLCVGPATEALAAEGLIVTAGGIDTHIHFI 120

Query: 136 PQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLEAAEEYAMNLGFLAKGNV 195
 PQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KGNV
 Sbjct: 121 PQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKGNV 180

Query: 196 S 196
S
Sbjct: 181 S 181

tr Q5KYM1 Urease alpha subunit (Urea amidohydrolase) (EC 3.5.1.5) 569
Q5KYM1_GEOKA [ureC] AA
[Geobacillus kaustophilus] align

Score = 286 bits (733), Expect = 2e-76
Identities = 144/244 (59%), Positives = 172/244 (70%), Gaps = 3/244 (1%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYE- 59
++Y M+GPTTGD +RL DTDL +E+EHDT YG+E+KFGGGK IRDGM Q + E
Sbjct: 8 RQYADMFGPTTGDCIRLADTDLWIEIEHDYTVYGDEVKFGGGKVIRDMGQHPLATRDEA 67

Query: 60 LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEG 119
+DLVLTNA+IVDYT + DGV N+ +G +TE +AAEG
Sbjct: 68 VDLVLTNAVIVDYTGIIYKADIGIKDGNIAAIGKAGNPLLMGCV--NIVIGASTEVIAAEG 125

Query: 120 LIVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
IVTAGG+D NHHFI PQOI TA +SG+TTMIGGGTGPA GTNATT TPG N+ ML A
Sbjct: 126 KIVTAGGVDAHIHFICPQQIETALSSGITTIGGGTGPATGTNATTCTPGEWNIYRMLEA 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
AE + MN+GFL KGN S + + +Q+ AGAIG K+HEDWG+T AAI CL VADEYDVQV
Sbjct: 186 AEAFFPMNIGFLGKGNASAKEPIAEQVRAGAIGLKLHEDWGTTAAIDAACLRVADEYDVQV 245

Query: 240 AIHT 243
AIHT
Sbjct: 246 AIHT 249

tr Q8GLB4 UreB (Fragment) [Helicobacter heilmannii] 181 AA
Q8GLB4_HELHE align

Score = 286 bits (733), Expect = 2e-76
Identities = 144/181 (79%), Positives = 151/181 (82%)

Query: 17 LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXX 76
LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QINSFSS+ELDLN+TNALIVDYT
Sbjct: 1 LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHELDLVITNALIVDYTGIIY 60

Query: 77 XXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGLIVTAGGIDTHIHFISSP 136
+QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISSP
Sbjct: 61 KADIGIKNGKIHGIGKAGNKDLQDGVNRLCVGPATEALAAEGLIVTAGGIDTHIHFISSP 120

Query: 137 QQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVS 196
QQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KGNVS
Sbjct: 121 QQIPTAFASGITTIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKGNVS 180

Query: 197 Y 197
+
Sbjct: 181 F 181

tr Q62HS0 Urease, alpha subunit (EC 3.5.1.5) [ureC] [Burkholderia] 568
 Q62HS0_BURMA mallei AA
 (Pseudomonas mallei)] align

Score = 285 bits (728), Expect = 8e-76
 Identities = 144/243 (59%), Positives = 173/243 (70%), Gaps = 2/243 (0%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
 + Y MYGPTTGDR+RL DT+L++EVE D T YGEE+KFGGGK IRDGM Q+ P++
 Sbjct: 8 RAYAEMYGPTTGDRIRLADTELLIEVERDHTLYGEEVKFGGGKVIRDGMGSQLPAADVA 67

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
 D V+TNA+I+D+ +Q GV + +G ATE +A EGL
 Sbjct: 68 DTVITNAVILDHWGIVKADIAIKHGRIAAIGKAGNPDIQPGV--TIAIGAATEIIAGEGL 125

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 IVTAGGIDTHIHFI SPQQI A ASGVTTMIGGGTGPA GTNATT TPG +++ ML+AA
 Sbjct: 126 IVTAGGIDTHIHFI SPQQIDEALASGVTTMIGGGTGPATGTNATTCTPGPWHMERMLQAA 185

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 + + +NLGFL KGN S L +QIEAGAIG K+HEDWG+TPAAI +CL VAD+ D QVA
 Sbjct: 186 DGWPINLGLGKGNASRPQPLVEQIEAGAIGLKLHEDWGTTPAIDNCLTVADDDTDQVA 245

Query: 241 IHT 243
 IHT
 Sbjct: 246 IHT 248

tr Q63RL3 Urease alpha subunit (EC 3.5.1.5) [ureC] [Burkholderia] 568
 Q63RL3_BURPS pseudomallei AA
 (Pseudomonas pseudomallei)] align

Score = 285 bits (728), Expect = 8e-76
 Identities = 144/243 (59%), Positives = 173/243 (70%), Gaps = 2/243 (0%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
 + Y MYGPTTGDR+RL DT+L++EVE D T YGEE+KFGGGK IRDGM Q+ P++
 Sbjct: 8 RAYAEMYGPTTGDRIRLADTELLIEVERDHTLYGEEVKFGGGKVIRDGMGSQLPAADVA 67

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGL 120
 D V+TNA+I+D+ +Q GV + +G ATE +A EGL
 Sbjct: 68 DTVITNAVILDHWGIVKADIAIKHGRIAAIGKAGNPDIQPGV--TIAIGAATEIIAGEGL 125

Query: 121 IVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 IVTAGGIDTHIHFI SPQQI A ASGVTTMIGGGTGPA GTNATT TPG +++ ML+AA
 Sbjct: 126 IVTAGGIDTHIHFI SPQQIDEALASGVTTMIGGGTGPATGTNATTCTPGPWHMERMLQAA 185

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 + + +NLGFL KGN S L +QIEAGAIG K+HEDWG+TPAAI +CL VAD+ D QVA
 Sbjct: 186 DGWPINLGLGKGNASRPQPLVEQIEAGAIGLKLHEDWGTTPAIDNCLTVADDDTDQVA 245

Query: 241 IHT 243
 IHT

Sbjct: 246 IHT 248

tr Q5FB23 Urease B subunit [ureB] [Campylobacter lari] 565 AA
Q5FB23_CAMLA align

Score = 285 bits (728), Expect = 8e-76

Identities = 147/243 (60%), Positives = 172/243 (70%), Gaps = 1/243 (0%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60

K+YV+MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGCK IRDGM+Q+ S +

Sbjct: 7 KDYVNMYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFF- 65

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGL 120

DLVLTNALIVDYT +QDQVD +L +G T+ + AEGL

Sbjct: 66 DLVLTNALIVDYTGIIKADIGIKNGYIVGIGKAGNPDIQDGVDPSTLIIGTNTDIIGAEGL 125

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180

IVTAGGIDTHIHFISSP QI A SGVTTMIGGG GP++GTNATT T S ++ SML+A

Sbjct: 126 IVTAGGIDTHIHFISSPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKAT 185

Query: 181 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHCLNVADEYDVQVA 240

+ Y MN GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVA

Sbjct: 186 QNYPMNFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVA 245

Query: 241 IHT 243

IHT

Sbjct: 246 IHT 248

tr Q8GLB3 UreB (Fragment) [Helicobacter heilmannii] 184 AA
Q8GLB3_HELHE align

Score = 283 bits (725), Expect = 2e-75

Identities = 142/180 (78%), Positives = 150/180 (82%)

Query: 21 DLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXXXXXX 80

DLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+ELDLV+TNALIVDYT

Sbjct: 3 DLILEVEHDCTTYGEEIKFGGGKTIRDGMGTNSPSSHELDLVITNALIVDYTGIIKADI 62

Query: 81 XXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISSPQQIP 140

+QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISSPQQIP

Sbjct: 63 GIKNGKIHGIGKAGNKDLQDGVNRLCVGPATEALAAEGLIVTAGGIDTHIHFISSPQQIP 122

Query: 141 TAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAMNLGFLAKGNVSYEPS 200

TAFASG+TTMIGGGTGPADGTNATTITPGR NLK MIRA+EYAMNLG+L KGNVS+EP+

Sbjct: 123 TAFASGITTMIGGGTGPADGTNATTITPGRWNKLEMLRASEEYAMNLGYLGKGNVSFEPA 182

tr Q8XXT1 PROBABLE UREASE (ALPHA SUBUNIT) PROTEIN (EC 3.5.1.5) 572
Q8XXT1_RALSO [ureC] AA
[Ralstonia solanacearum (Pseudomonas solanacearum)] align

Score = 282 bits (722), Expect = 4e-75
Identities = 145/243 (59%), Positives = 169/243 (68%), Gaps = 2/243 (0%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYEL 60
+ Y M+GPTTGDRVRL DTDLI+EVE D T YGEE+KFGGGK IRDGM Q+ S
Sbjct: 8 RAYAEMFGPTTGDRVRLADTDLIVEVERDYTIYGEVVKFGGGKVIRDMGMSQRESKDCA 67

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
D V+TNALI+D+ +Q GV + +GP TE +A EG+
Sbjct: 68 DTVITNALIIDHWGIVKADIGLKHGRIAAIGKAGNPDIQPGV--TIVIGPGTEIIAGEGM 125

Query: 121 IVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAA 180
IVTAGG+DTHIHFI PQQI A SGVTTMIGGGTGPA GT ATT TFG +L+ ML+AA
Sbjct: 126 IVTAGGVDTHIHFIQIQIDEALNSGVTTMIGGGTGPATGTATTCTPGPWHLQRLQAA 185

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHCLNVADEYDVQVA 240
+ Y MN+GFL KGN S +LR+QI+AGAIG K+HEDWGSTPAAI CL VAD+ D QVA
Sbjct: 186 DAYPMNIGFLGKNGSLPGALREQIDAGAIGLKLHEDWGSTPAAIDCCLGVADDTDTQVA 245

Query: 241 IHT 243
IHT
Sbjct: 246 IHT 248

tr Q8GLB6 UreB (Fragment) [Helicobacter heilmannii] 180 AA
Q8GLB6_HELHE align

Score = 282 bits (721), Expect = 5e-75
Identities = 141/180 (78%), Positives = 150/180 (83%)

Query: 21 DLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYELDLVLTNALIVDYTXXXXXXXX 80
DLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPS+EELDV+TNALIVDYT
Sbjct: 1 DLILEVEHDCTTYGEEIKFGGGKTIRDGMQTNSSSHELDLVITNALIVDYTGIIYKADI 60

Query: 81 XXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGLIVTAGGIDTHIHFISSPQQIP 140
+QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISSPQQIP
Sbjct: 61 GIKNGKIHGIGKAGNKDLQDGVNRLCVGPATEALAAEGLIVTAGGIDTHIHFISSPQQIP 120

Query: 141 TAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEEYAMNLGFLAKGNVSYEPS 200
TAFASG+TTMIGGGTGPADGTNATTITPGR NLE MLRA+EEYAMNLG+L +GNVS+EP+
Sbjct: 121 TAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGQGNVSFEPA 180

tr Q8GLB2 UreB (Fragment) [Helicobacter heilmannii] 178 AA
Q8GLB2_HELHE align

Score = 281 bits (720), Expect = 6e-75
Identities = 141/178 (79%), Positives = 148/178 (82%)

Query: 16 RLGDSDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYELDLVLTNALIVDYTX 75
RLGDSDL+LEVEHDCTTYGEEIKFGGGKTIRDGM QTNSSPS+EELDV+TNALIVDYT
Sbjct: 1 RLGDSDLVLEVEHDCTTYGEEIKFGGGKTIRDGMQTNSSSHELDLVITNALIVDYTGII 60

Query: 76 XXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGLIVTAGGIDTHIHFISS 135
+QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISS

Sbjct: 61 YKADIGIKNGKIHGIGKAGNKDLQDGVNRLCVGPATEALAAEGLIVTAGGIDTHIHFIS 120

Query: 136 PQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEEYAMNLGFLAKG 193
PQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KG

Sbjct: 121 PQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKG 178

tr Q8YQZ0 Urease alpha subunit [alr3670] [Anabaena sp. (strain PCC 568
Q8YQZ0_ANASP 7120)] AA
[align](#)

Score = 281 bits (718), Expect = 1e-74
Identities = 144/242 (59%), Positives = 170/242 (69%), Gaps = 3/242 (1%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELD 61
Y YGPT GDR+RL DT+L ++VE D TTYG+E+KFGGKK IRDGM Q+ + + +D

Sbjct: 10 YAETYGPTVGDRIRLADTELFIQVEQDFTTYGDEVKFGGGKVIRDGMGQSPIANADGAVD 69

Query: 62 LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNRLCVGPATEALAAEGLI 121
LV+TNALI+D+ +QD VD + +GF TEALA EG+I

Sbjct: 70 LVITNALILDWWGIVKADIGIKDGKIFKIGKAGNPYIQDHVD--IIIGPGTEALAGEGMI 127

Query: 122 VTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAE 181
+TAGGIDTHIHF I PQQI A ASG+TTMIGGGTGPA GTNATT TPG N+ ML+AA+

Sbjct: 128 LTAGGIDTHIHFICPQQIEVAIASGITTMIGGGTGPATGTNATTCTPGPWNMYRMLQAAD 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
+ MNLGFL KGN S L +QI AGAIG K+HEDWG+TPA I CL VADEYDVQVAI

Sbjct: 188 AFPMNLGFLGKGNASQPQGLVEQIFAGAIGLKLHEDWGTTPATIDTCLTVADEYDVQVAI 247

Query: 242 HT 243
HT

Sbjct: 248 HT 249

tr Q6I6H5 Urease beta subunit (Fragment) [ureB] [Campylobacter 454
Q6I6H5_CAMLA lari] AA
[align](#)

Score = 280 bits (716), Expect = 2e-74
Identities = 144/238 (60%), Positives = 169/238 (70%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELDLVLT 65
MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGKK IRDGM+Q+ S + DLVLT

Sbjct: 1 MYGPTTNDRVRLADTDLILRVEKDYTTYGEEVKFGGGKNIRDGMAQSVSEGDFF-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNRLCVGPATEALAAEGLIVTAG 125
NALIVDYT +QDGVDT+L +G +T+ + AEGIVTAG

Sbjct: 60 NALIVDYTGIIYKASIGIKNGYIVGIGKAGNPDIQDGVDSLLIIGTSTDIIGAEGIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEEYAM 185
GIDTHIHFISP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+AA + Y M

Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSLMKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243

N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT
 Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIHT 237

tr Q6I6I9 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
 Q6I6I9_CAMLA lari] AA
align

Score = 278 bits (712), Expect = 5e-74
 Identities = 144/238 (60%), Positives = 168/238 (70%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLT 65
 MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S + DLVLT
 Sbjct: 1 MYGPTTNDVRRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAG 125
 NALIVDYT +QDQVD +L +G +T+ + AEGGLIVTAG
 Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPGLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
 GIDTHIHFISSP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+A + Y M
 Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
 N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT
 Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIHT 237

tr Q6I6H3 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
 Q6I6H3_CAMLA lari] AA
align

Score = 278 bits (712), Expect = 5e-74
 Identities = 144/238 (60%), Positives = 168/238 (70%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLT 65
 MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S + DLVLT
 Sbjct: 1 MYGPTTNDVRRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAG 125
 NALIVDYT +QDQVD +L +G +T+ + AEGGLIVTAG
 Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPGLIIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
 GIDTHIHFISSP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+A + Y M
 Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
 N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT
 Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVAIHT 237

tr Q6I6I3 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454

Q6I6I3_CAMLA lari]

AA
align

Score = 278 bits (711), Expect = 7e-74

Identities = 144/238 (60%), Positives = 167/238 (69%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLT 65
 MYGPTT DRVRL DTDLLI VE D T YGEE+KFGGGK IRDGM+Q+ S + LDVLT
 Sbjct: 1 MYGPTTNDVRRLADTDLILRVEKDYTYGEEVKFGGGKNIRDGMAQSVSEGDF-LDLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGLIVTAG 125
 NALIVDYT +QDQVD +L +G +T+ + AEGGLIVTAG
 Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPVLVIGTSTDIIGAEGGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
 GIDTHIHFISSP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+A Y M
 Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKATHNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
 N GFL KGN S + +L++QI AGA G KIHEDWG+T + I L +ADE D+QVAIHT
 Sbjct: 180 NFGFLGKGNSSNKNALKEQITAGACGLKIHEDWGATSSVIDASLKIADMDIQVAIHT 237

tr Q7V3V2 Urease alpha subunit (EC 3.5.1.5) [ureC] 574
 Q7V3V2_PROMM [Prochlorococcus marinus] AA
 (strain MIT 9313) align

Score = 277 bits (709), Expect = 1e-73

Identities = 142/242 (58%), Positives = 169/242 (69%), Gaps = 3/242 (1%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELD 61
 Y YGPTTGDR+RL DT+LILEVE D TTYGEE+KFGGGK IRDGM Q+ S ++ +D
 Sbjct: 10 YAETYGPTTGDRIRLADTELILEVERDFTTYGEEVKFGGGKVIRDGMGQSQQSRANGAVD 69

Query: 62 LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGLI 121
 V+TNALI+D+ + DG+D + +GP TEA+A EG I
 Sbjct: 70 TVITNALILDWWGIVKADIGLRDGRIVAIGKAGNPDIITDGID--IVIGPGTEAIAEGHI 127

Query: 122 VTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
 VTAG ID+HINFI PQI EA ASGVTTM+GGGTGPA GTNATT TPG ++ ML+AAE
 Sbjct: 128 VTAGAIDSHIHFISSPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMLQAAE 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
 MNLGE KGN S +L +Q+ AGA G K+HEDWG+TPAAI CL+VAD +DVQV I
 Sbjct: 188 GLPMNLGFFGKGNASTAEALEEQVLAGACGLKLHEDWGTTTAAIDCCLSVADRFVQVCI 247

Query: 242 HT 243
 HT
 Sbjct: 248 HT 249

tr Q6I6J1 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
 Q6I6J1_CAMLA lari] AA
align

Score = 277 bits (709), Expect = 1e-73
Identities = 144/238 (60%), Positives = 167/238 (69%), Gaps = 1/238 (0%)

```
Query: 6  MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLT 65
          MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S + DLVLT
Sbjct: 1  MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFF-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGLIVTAG 125
          NALIVDYT +QDGV +L +G T+ + AEGGLIVTAG
Sbjct: 60 NALIVDYTGIIKADIGIKNGYIVGIGKAGNPDIQDGVDPSTLIIGTNTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
          GIDTHIHFISSP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+A + Y M
Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
          N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMIDIQVAIHT 237
```

tr Q6I6I5 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
Q6I6I5_CAMLA lari] AA
align

Score = 277 bits (708), Expect = 2e-73
Identities = 143/238 (60%), Positives = 167/238 (70%), Gaps = 1/238 (0%)

```
Query: 6  MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLT 65
          MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S + DLVLT
Sbjct: 1  MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFF-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEGLIVTAG 125
          NALIVDYT +QDGV +L +G +T+ + AEGGLIVTAG
Sbjct: 60 NALIVDYTGIIKADIGIKNGYIVGIGKAGNPDIQDGVDPSTLIIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
          GIDTHIHFISSP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+ + Y M
Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
          N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMIDIQVAIHT 237
```

tr Q6I6H1 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
Q6I6H1_CAMLA lari] AA
align

Score = 276 bits (707), Expect = 2e-73
Identities = 142/238 (59%), Positives = 167/238 (69%), Gaps = 1/238 (0%)

```
Query: 6  MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDLVLT 65
          MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S + DLVLT
Sbjct: 1  MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFF-DLVLT 59
```

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAG 125
 NALIVDYT +QDQVD +L +G +T+ + AEGLI+TAG
 Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPVLVIGTSTDIIGAEGLIITAG 119

Query: 126 GIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
 GIDTHIHFIISP QI A SGVTTMIGGG GP++GTNATT T G ++ SML+ + Y M
 Sbjct: 120 GIDTHIHFIISPQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMKLTQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
 N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT
 Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADMDIQVAIHT 237

tr Q5E728 Urease alpha subunit (EC 3.5.1.5) [VF0673] [Vibrio 567
 Q5E728_VIBf1 fischeri (strain AA
 ATCC 700601 / ES114)] align

Score = 276 bits (707), Expect = 2e-73
 Identities = 138/241 (57%), Positives = 169/241 (69%), Gaps = 2/241 (0%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
 Y +M+GPTTGDR+RL DT+L LEVE D TTYGEE+KFGGGK IRDGM Q+ +S +D+
 Sbjct: 9 YANMFGPTTGDRRLRLADTELFLEVEKDFTTYGEEVKFGGGKVIRDGMGQSQVVNSECVDV 68

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIV 122
 V+TNALI+D+ +Q VD + VGPATE +A EG I+
 Sbjct: 69 VITNALILDHWGIVKADIGIKDGRIFGIGKAGNPDPVQPNVD--IVVGPATEVVAGEGKII 126

Query: 123 TAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
 TAGG+DTHIHFI PQQ SGVTT IGGGTGP GTNATT+TPG N+ ML A ++
 Sbjct: 127 TAGGVDTHIHFIICPQQAEEGLTSGVTTFIGGGTGPVAGTNATTVTPGIWNMHRMLEAVDD 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
 +N+G KG VS +LR+QIEAGA+S KIHEDWG+TPAAIH+CLNVAE D+Q+AIH
 Sbjct: 187 LPINVGLFGKGCVSKPEALREQIEAGAMGLKIHEDWGATPAAIHCLNVADEMIDIQIAIH 246

Query: 243 T 243
 +
 Sbjct: 247 S 247

tr Q8DMV6 Urease alpha subunit [ureC] [Synechococcus elongatus 572 AA
 Q8DMV6_SYNEL (Thermosynechococcus elongatus)] align

Score = 276 bits (705), Expect = 4e-73
 Identities = 136/242 (56%), Positives = 172/242 (70%), Gaps = 3/242 (1%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSY-ELD 61
 Y YGPT GDR+RL DTDLI+E+END T YG+E+KFGGGK IRDGM Q+ ++ +D
 Sbjct: 10 YAETYGPTVGDRLRLADTDLIIEIEHDYTHYGDEVKFGGGKVIRDGMGQSPIANAEGAVD 69

Query: 62 LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLI 121
 +V+TNA+I+D+ Q+GVD + +GF TEA+A EG+I
 Sbjct: 70 VVITNAVILDWVGWVKADVGIKDKIYKIGKAGNPYTQEGVD--IIIGPGTEAIAEGMI 127

Query: 122 VTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAE 181
+TAGGID HINFI PQQI TA A+G+TTMIGGGTGPA GTNATT TPG N+ ML+AA+
Sbjct: 128 LTAGGIDAHIHFI CPQQIATAIAAGITTMIGGGTGPATGTNATTCTPGPWNIYRMLQAAD 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
+ +NLGFL KGN S L +Q++AG +G K+HEDWGSTP AI CL+VA++YD+QVAI
Sbjct: 188 AFPVNLGFLGKGNSSQPQGLIEQVQAGVVGLKLHEDWGSTPNAIDTCLSAEDYDIQVAI 247

Query: 242 HT 243
RT
Sbjct: 248 HT 249

tr Q4ZN06 Urease (EC 3.5.1.5) [Psynr_4436] [Pseudomonas syringae] 566
Q4ZN06_PSESY pv. syringae AA
B728a] align

Score = 275 bits (702), Expect = 8e-73
Identities = 137/241 (56%), Positives = 170/241 (69%), Gaps = 2/241 (0%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDL 62
Y M+GPT GD+VRL DT+L +EVE D TTYGEE+KFGGGK IRDGM Q ++ +D
Sbjct: 8 YADMFGPTVGDKVRLADTELWIEVEKDFTTYGEEVKFGGGKVIRDGMGQGQLLAAEVVD 67

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIV 122
+++TNALI+D+ +Q V + VG ATE +A EG+I+
Sbjct: 68 LITNALIIDHWGIVKADVGIKNGRIAAIGKAGNPDIQPDV--TIAVGAATEVIAGEGMIL 125

Query: 123 TAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEE 182
TAGG+DTHIHFI PQQI A SGVTTMIGGGTGPA GTNATT+TPG ++ ML+A++
Sbjct: 126 TAGGVDTHIHFI CPQQIEEALMSGVTTMIGGGTGPATGTNATTVTGPWHMARMLQASDS 185

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
+ MN+GF KGNVS L +Q++AGAIG K+HEDWG+TEAAI +CL+VADEYDVQVAIH
Sbjct: 186 FPMNIGFTGKGNVSLPGPLIEQVKAGAIGLKLHEDWGTTTAAIDNCLSVADYDVQVAIH 245

Query: 243 T 243
T
Sbjct: 246 T 246

tr Q9RYJ4 Urease, alpha subunit [DRA0318] [Deinococcus] 568
Q9RYJ4_DEIRA radiodurans] AA
align

Score = 274 bits (701), Expect = 1e-72
Identities = 135/246 (54%), Positives = 171/246 (68%), Gaps = 3/246 (1%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYEL 60
++Y +YGPT GDRVRLGDT+L++EVE D TTYGEE+KFGGGK IRDGM Q+++ + +
Sbjct: 6 QQYADLYGPTVGDRVRLGDTTELLIEVERDLTTYGEEVKFGGGKVIRDGLGQSSAATRDDA 65

Query: 61 ---DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAA 117
DLV+TNALI+DY QDRV L + +TE +A

Sbjct: 66 NVPDLVITNALILDYWGVIKADVGVKNGRISAIGKAGNPGTQDGVTPGLTIAASTEIVAG 125

Query: 118 EGLIVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSM 177
 EGL++TAGG+DTHIHFI+PQQ TA SGVTTMIGGGTGP GT+ATT TPG+ ++ ML

Sbjct: 126 EGLVLTAGGVDTHIHFIAPQQCWTALESGVTTMIGGGTGPTAGTSATTCTPGQWHIHRML 185

Query: 178 RAAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDV 237
 + +N G L KGN S +P L +QI AGA+G K+HEDWG+TPAAIH L+VA++YDV

Sbjct: 186 ESLAGLPLNFGLLGKGNASTQPPLAEQIRAGALGLKLHEDWGTTPAAIHAALSVAEDYDV 245

Query: 238 QVAIHT 243
 QVAIHT

Sbjct: 246 QVAIHT 251

tr Q87VP0 Urease, alpha subunit [ureC] [Pseudomonas syringae (pv. 566
 Q87VP0_PSESM tomato)] AA
align

Score = 274 bits (701), Expect = 1e-72
 Identities = 137/241 (56%), Positives = 170/241 (69%), Gaps = 2/241 (0%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDL 62
 Y M+GPT GD+VRL DT+L +EVE D TTYGEE+KFGGGK IRDGM Q ++ +D

Sbjct: 8 YADMFGPTVGDKVRLADTELWIEVEKDFTTYGEEVKFGGGKVIRDGMGQGQLLAADVDT 67

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIV 122
 ++TNALI+D+ +Q V + VG ATE +A EG+I+

Sbjct: 68 LITNALIIDHWGIVKADVGIKNGRIAAIGKAGNPDIQPDV--TIAVGAATEVIAGEGMIL 125

Query: 123 TAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEE 182
 TAGG+DTHIHFI PQQI A SGVTTMIGGGTGP A GTNATT+TPG ++ ML+A++

Sbjct: 126 TAGGVDTHIHFIQPPQIEEALMSGVTTMIGGGTGPATGTNATTVTGPGWHMARMLQASDS 185

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
 + MN+GF KGNVS L +Q++AGAIG K+HEDWG+TEAAI +CL+VADEYDVQVAIH

Sbjct: 186 FPMNIGFTGKGNVSLPGPLIEQVKAGAIGLKLHEDWGTTPAIDNCLSVADYDVQVAIH 245

Query: 243 T 243
 T

Sbjct: 246 T 246

sp P16122 Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) 567
 URE1_PROVU [ureC] AA
 [Proteus vulgaris] align

Score = 274 bits (700), Expect = 1e-72
 Identities = 135/241 (56%), Positives = 166/241 (68%), Gaps = 2/241 (0%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSPYELDL 62
 Y M+GPTTGD+RL DT+L LE+E D TTYGEE+KFGGGK IRDGM Q+ S+ +D+

Sbjct: 9 YADMFGPTTGDRRLRLADTELFLEIEQDFTTYGEEVKFGGGKVIRDGMGQSQVVSACVDV 68

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIV 122

```

++TNA+I+D+          +Q VD + +GP TE +A EG I+
Sbjct: 69 LITNAIIIDHWGIVKADIGIKDGRITGIGKAGNPDVQPNVD--IVIGPGTEVVAGEGKII 126

Query: 123 TAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEE 182
          TAGG+DTHIHFI PQQ          SGVTT IGGGTGP GTNATT+TPG N+ ML A +E
Sbjct: 127 TAGGVDTHIHFIQQAEEGLISGVTTFIGGGTGPVAGTNATTVTPTGIWNMHRMLEAVDE 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
          +N+G KG VS ++R+QIEAGAIG KIHEDWG+TP ALH+CLNVADE DVQVAIH
Sbjct: 187 LPINVGLFGKGCVSQPEAIREQIEAGAIGLKIHEWDGATPMAIHNCLNVADEMVDVQVAIH 246

Query: 243 T 243
          +
Sbjct: 247 S 247

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tr Q733J6      Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacillus      570
Q733J6_BACC1  cereus (strain                                     AA
               ATCC 10987)]                                       align

```

Score = 273 bits (698), Expect = 2e-72
Identities = 134/245 (54%), Positives = 171/245 (69%), Gaps = 4/245 (1%)

```

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYE- 59
          K+Y +YGPTTGD +RL DT L +E + T YG+E FGGGK+IRDGM Q + + +
Sbjct: 8 KQYADLYGPTTGDSIRLADTQLFAHIERNATVYGDEAVFGGGKSIRDGMGQNSQLTREQG 67

Query: 60 -LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAE 118
          +D+V+TNA+I+DYT          + D +D + +G +TE ++ E
Sbjct: 68 VVDVVITNAIIIDYTGIYKADIGIKDGKISAIGKSGNPSVMDNID--IIIGTSTEVISGE 125

Query: 119 GLIVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLR 178
          IVTAGGIDTH+HFIISPQQI TA ASG+TT+IGGSTGPA+GT ATTITPG NL+ ML
Sbjct: 126 RKIVTAGGIDTHVHFISPQQIDTALASGITTLIGGGTGPAGETKATTITPGSWNLRKMLE 185

Query: 179 AAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQ 238
          AAEE + +NLGFL EGN S P+L +QI AGAIG KIHEDWG+T +AI+H L +AD+YD+Q
Sbjct: 186 AAFAFPINLGLGKGNSSSLPALEEQIFAGAIGLKIHEWDGATSSAINHSLQIADKYDIQ 245

Query: 239 VAIHT 243
          VAIHT
Sbjct: 246 VAIHT 250

```

```

sp P17086      Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) 567
URE1_PROMI    [ureC]                                                  AA
               [Proteus mirabilis]                                    align

```

Score = 273 bits (697), Expect = 3e-72
Identities = 137/241 (56%), Positives = 165/241 (67%), Gaps = 2/241 (0%)

```

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYELD 62
          Y M+GPTTGD+RL DT+L LE+E D TTYGEE+KFGGGK IRDGM Q+ S+ +D+
Sbjct: 9 YADMFGPTTGDRRLRLADTELFLEIEKDFTTYGEEVKFGGGKVIRDGMGQSQVVSACVDV 68

```

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIV 122
 ++TNA+I+DY +Q VD + +GP TE +A EG IV
 Sbjct: 69 LITNAIILDYWGIVKADIGIKDGRIVGIGKAGNPVQPNVD--IVIGPGTEVVAGEGKIV 126

Query: 123 TAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 182
 TAGGIDTHIHFI PQQ SGVTT IGGGTGP GTNATT+TPG N+ ML A +E
 Sbjct: 127 TAGGIDTHIHFI CPQQAQEGLVSGVTTFFIGGGTGPVAGTNATTVTPIWNMYRMLEAVDE 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
 +N+G EG VS ++R+QI AGAIG KIHEDWG+TP ALH+CLNVADE DVQVAIH
 Sbjct: 187 LPINVGLFGKGCVSQPEAIREQITAGAIGLKI HEDWGATPMAIHNCLNVADEMDVQVAIH 246

Query: 243 T 243
 +
 Sbjct: 247 S 247

sp P73061 Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) 569
 URE1_SYNY3 [ureC] AA
 [Synechocystis sp. (strain PCC 6803)] align

Score = 272 bits (696), Expect = 4e-72
 Identities = 136/242 (56%), Positives = 173/242 (71%), Gaps = 3/242 (1%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELD 61
 Y +GPT GD+VRL DT+L +EVE D TYG+E+KFGGGK IRDGM Q+ S + +D
 Sbjct: 10 YAHTFGPTVGDKVRLADTELFIEVEQDYATYGDVKGFGGGKVIRDGMGQSPLSRAEAVD 69

Query: 62 LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLI 121
 +V+TNALI+D+ +QD V ++ +GP+TEA+A EG+I
 Sbjct: 70 VVITNALILDWWGIVKADVGIKNGRIYAIGKAGNPHIQDNV--SIIIGPSTEAIAGEGMI 127

Query: 122 VTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
 +TAGSID H+HEI PQQI TA ASGVTT++GGGTGPA GT ATT TPG N+ ML+AA+
 Sbjct: 128 LTAGGIDAHVHFICPQQIETALASGVTTLVGGGTGPAAGTKATTCTPGAWNIHRLQAAD 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
 + +NLGEL KGN S +L +QI+AGAIG K+HEDWG+TPAAI +CL VA++YDVQVAI
 Sbjct: 188 GFPINLGFLGKNGSQPAALAEQIKAGAILKLHEDWGTTPAIDNCLGVAEDYDVQVAI 247

Query: 242 HT 243
 HT
 Sbjct: 248 HT 249

tr Q7U3I3 Urease alpha subunit (EC 3.5.1.5) [ureC] [Synechococcus] 569
 Q7U3I3_SYNPX sp. (strain WH8102)] AA
align

Score = 272 bits (696), Expect = 4e-72
 Identities = 141/242 (58%), Positives = 165/242 (67%), Gaps = 3/242 (1%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSY-ELD 61
 Y YGPTTGDRVRL DEDLILEVE D T YG+E+KFGGGK IRDGM Q+ +P + +D
 Sbjct: 10 YAETYGPTTGDRVRLADTDLILEVEKDYTVYGDEVKFGGGKVIRDGMGQSQTPTREGAVD 69

Query: 62 LVLVTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLI 121
 V+TNALI+D+ Q GV + VGP TEA+A EG I
 Sbjct: 70 TVITNALILDWWGIVKADVGLKDGRIVGIGKAGNPDTQQGV--TIVVGPTEAIAGEGHI 127

Query: 122 VTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
 +TAGGIDTHIHFI PQQI TA ASGVTT++GGGTGPA GINATT TPG ++ ML+AAE
 Sbjct: 128 LTAGGIDTHIHFIICPQQIETALASGVTTLMGGGTGPATGTNATTCTPGAFHIGRMLQAAE 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
 +NLGF KGN S +L +Q+ AGA G K+HEDWG+TPA I CL+VAD DVQV I
 Sbjct: 188 GLPVNLGFFGKGNASTPEALEEQVRAGACGLKLHEDWGTTTPATIDACLSVADRMVDQVCI 247

Query: 242 HT 243
 HT
 Sbjct: 248 HT 249

tr 052305 Urease alpha subunit [ureC] [Synechococcus sp. (strain 569
 052305_SYN2 PCC 7002) AA
 (Agmenellum quadruplicatum)] align

Score = 272 bits (696), Expect = 4e-72
 Identities = 137/244 (56%), Positives = 169/244 (69%), Gaps = 3/244 (1%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSP-SSYE 59
 + Y YGPT GDR+RL DT+L LEVE D TTYG+E+KFGGOK IRDGM Q+ P +
 Sbjct: 8 RAYAETYGPTVGDRLRLADTELWLEVEQDFTTYGDEVKFGGGKVIRDMGQSAVPRADGA 67

Query: 60 LDLVLVTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEG 119
 +D V+TNALI+D+ +QD +D + +GP TEA+A EG
 Sbjct: 68 VDTVITNALIVDWWGIVKADVGIKDGKIYKIGKAGNPDIQDNID--IIIGPGTEAIAGEG 125

Query: 120 LIVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
 I+TAGGID+H+RFL PQQI A ASG+TMIGGGTGP GINATT TPG ++ ML A
 Sbjct: 126 HILTAGGIDSHVHFICPQQIEVAIASGITTMIGGGTGPATGTNATTCTPGEWIHISMILEA 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
 AE + +NLGF KGN + L +Q+ AG IG K+HEDWG+TPAAI +CL+VAD++DVQV
 Sbjct: 186 AEGFPINLGFTGKNSAKPEGLIEQVRAGVIGLKLHEDWGTTTAAIDNCLSVADQFDVQV 245

Query: 240 AIHT 243 -
 AIHT
 Sbjct: 246 AIHT 249

tr Q6I6H7 Urease beta subunit (Fragment) [ureB] [Campylobacter 454
 Q6I6H7_CAMLA lari] AA
align

Score = 272 bits (695), Expect = 5e-72
 Identities = 141/238 (59%), Positives = 166/238 (69%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYELDLVLT 65
 MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGOK IRDGM+Q+ S + DLVLT

Sbjct: 1 MYGPTTNDVRVLADTDLILRVEKDYTLYGEEVKFGGKGNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAG 125
NALIVDYT +QDQVD +L +G +T+ + AEGGLIVTAG

Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSSLVIGTSTDIIGAEGGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
GIDTHIHFISSP QI A SGVTTMIG GP++GTNATT T G ++ SML++ + Y M

Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGARIGPSEGTNATTCTSGAYHIHSMMLKSTQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAIHT

Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMIDIQVAIHT 237

tr Q6I6I1 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
Q6I6I1_CAMLA lari] AA
align

Score = 271 bits (692), Expect = 1e-71
Identities = 140/238 (58%), Positives = 165/238 (68%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGKKTIRDGMSQTNSSPSYELDLVLT 65
MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGSK IRDGM+Q+ S + DLVLT

Sbjct: 1 MYGPTTNDVRVLADTDLILRVEKDYTLYGEEVKFGGKGNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAG 125
NALIVDYT +QDQVD +L +G +T+ + AEGGLIVTAG

Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSSLVIGTSTDIIGAEGGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185
GIDTHIHFISSP QI A SGVTTMIG GP++GTNATT T G ++ SML++ Y M

Sbjct: 120 GIDTHIHFISSPTQIECALYSGVTTMIGAPIGPSEGTNATTCTSGAYHIHSMMLKSTHNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+Q+AIHT

Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMIDIQIAIHT 237

tr Q6I6H9 Urease beta subunit (Fragment) [ureB] [Campylobacter] 454
Q6I6H9_CAMLA lari] AA
align

Score = 271 bits (692), Expect = 1e-71
Identities = 140/238 (58%), Positives = 165/238 (68%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGKKTIRDGMSQTNSSPSYELDLVLT 65
MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGSK IRDGM+Q+ S + DLVLT

Sbjct: 1 MYGPTTNDVRVLADTDLILRVEKDYTLYGEEVKFGGKGNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIVTAG 125
NALIVDYT +QDQVD +L +G +T+ + AEGGLIVTAG

Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSSLVIGTSTDIIGAEGGLIVTAG 119

Query: 126 GIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEYAM 185

GIDTHIHFIISP QI A SGVTTMIG GP++GENATT T G ++ SML++ Y M
 Sbjct: 120 GIDTHIHFIISPQIECALYSGVTTMIGAPIGPSEGTNATTCTSGAYHIHSMKSTHNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
 N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+Q+AIHT
 Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQIAIHT 237

tr Q6FD83 Urease alpha subunit (EC 3.5.1.5) [ureC] [Acinetobacter 566
 Q6FD83_ACIAD sp. (strain AA
 ADP1)] align

Score = 270 bits (691), Expect = 1e-71
 Identities = 135/243 (55%), Positives = 170/243 (69%), Gaps = 2/243 (0%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYEL 60
 + Y M+GPT GDRVRL DT+L +EVE D TTYGEE+KFGGGK IRDGM Q+ +
 Sbjct: 6 RAYAEMFGPTVGDRVRLADTELFIEVEQDLTTYGEEVKFGGGKVIRDGMGQSOLLADEVA 65

Query: 61 DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGL 120
 D V+TNALIVD+ +Q D + +G ATE +A EG
 Sbjct: 66 DTVITNALIVDWWGIVKADVGLKNGRIWKIGKAGNPDIQP--DITIPLGAATEVIAGEGQ 123

Query: 121 IVTAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
 I+TAGGIDTHIH+I PQQ+ TA SGVTTM+GGGTGPA GT+ATT+TEG ++ +ML+A
 Sbjct: 124 ILTAGGIDTHIHWICPQQVETALMSGVTTMVGGGTGPAAAGTSATTVTGPWHIGTMLQAI 183

Query: 181 EEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
 ++ MN+G L KGN+S +R+QI+AG +G K+HEDWGSTPAAT +CL+VADE+DVQVA
 Sbjct: 184 DDLPMNIGLLGKGNLSLPDPIREQIKAGVVGLKLHEDWGSTPAAIDNCLSVADEFDVQVA 243

Query: 241 IHT 243
 IHT
 Sbjct: 244 IHT 246

tr Q9HUU5 Urease alpha subunit [ureC] [Pseudomonas aeruginosa] 566 AA
 Q9HUU5_PSEAE align

Score = 270 bits (689), Expect = 3e-71
 Identities = 136/241 (56%), Positives = 165/241 (68%), Gaps = 2/241 (0%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNPSSEYELDL 62
 Y M+GPT GDRVRL DTDL +EVE D T YGEE+KFGGGK IRDGM Q+ ++ +D
 Sbjct: 8 YADMFGPTVGDRVRLADTDLWIEVERDFTVYGEEVKFGGGKVIRDGMGQSOLGAAQVVD 67

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNNLCVGPATEALAAEGLIV 122
 V+TNALI+D+ +Q GV N+ +G TE +A EG+I+
 Sbjct: 68 VITNALILDHWGVVKADVGLKDGRIQAIGKAGNPDIQPGV--NIAIGAGTEVIAGEGMIL 125

Query: 123 TAGGIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
 TAGGIDTHIHFI PQQI A SGVTTMIGGGTGPATNATT T G ++ ML+AA+
 Sbjct: 126 TAGGIDTHIHFICPQQIEEALMSGVTTMIGGGTGPAAAGTNATTCTSGPWHMARMQAADA 185

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
 + MN+GF KGN S L +Q+ AGAIG K+REDWGSTPAAL +CL VA+ +D+QVAIH
 Sbjct: 186 FPMNIGFTGKGNASLPLPLEEQVLAGAIGLKLHEDWGSTPAAIDNCLEVAERHDIQVAIH 245

Query: 243 T 243
 T
 Sbjct: 246 T 246

tr Q88J04 Urease, alpha subunit [ureC] [Pseudomonas putida (strain 567
 Q88J04_PSEPK KT2440)] AA
align

Score = 270 bits (689), Expect = 3e-71
 Identities = 140/241 (58%), Positives = 164/241 (67%), Gaps = 2/241 (0%)

Query: 3 YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSSSYELD 62
 Y M+GPT GDRVRL DT L +EVE D T YGEE+KFGGGK IRDGM Q ++ +DL
 Sbjct: 9 YADMFGPTVGDRLADTALWVEVEKDFTIYGEEVKFGGGKVIRDGMGQGMALAEAMD 68

Query: 63 VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEGLIV 122
 VLTNALI+D+ +Q GV N+ VGF TE +AAEG IV
 Sbjct: 69 VLTNALIIDHWGIVKADIGIKHGRIAVIGKAGNPDPVQPGV--NVPVPGPGTEVIAAEGKIV 126

Query: 123 TAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLAAEE 182
 TAGG+D+THIHF PQQ+ A SGVTT IGGGTGPA GTNATT TPG L ML+AA+
 Sbjct: 127 TAGGVDSHIHFICPQQVDEALNSGVTTFFIGGGTGPATGTNATTCTPGPWYLARMLQAADS 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
 +N+G L KGN S +LR+QI AGA+G K+REDWGSTPAAL CL VA+E D+QVAIH
 Sbjct: 187 LPINIGLLGKGNASRPDALREQIAAGAVGLKLHEDWGSTPAAIDCCLGVAEEMDIQVAIH 246

Query: 243 T 243
 T
 Sbjct: 247 T 247

tr Q7V1B6 Urease alpha subunit (EC 3.5.1.5) [ureC] 569
 Q7V1B6_PROMP [Prochlorococcus marinus AA
 subsp. pastoris (strain CCMP 1378 / MED4)] align

Score = 268 bits (686), Expect = 6e-71
 Identities = 136/244 (55%), Positives = 167/244 (67%), Gaps = 3/244 (1%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYE 59
 K Y YGPT GDRVRL DT+LI+EVE D TTYG+E+KFGGGK IRDGM Q+ +
 Sbjct: 8 KTYAQTYGPTKGDRLADTELIIEVEKDFTTYGDEVKFGGGKVIRDGMGQSQVTREDGA 67

Query: 60 LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDDNNLCVGPATEALAAEG 119
 +D V+TNALIVD+ +QD + N+ +G +TE +A EG
 Sbjct: 68 VDTVITNALIVDWWGIVKADVGLKDGKIYEIGKAGNPDIQDNI--NIIIGSSSTEVIAEG 125

Query: 120 LIVTAGGIDTHIHFISSPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLA 179
 L+TAG IDTHIHF PQQI TA ASGVTTM+GGGTGPA GTNATT TPG ++ M+++
 Sbjct: 126 HILTAGSIDTHIHFISSPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMIS 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
 AE + +NLGF KGN S E +L +Q+ AGA G K+HEDWG+TP+ I+ CLNVAD DVQV
 Sbjct: 186 AEAFPVNLGFFGKGNSSNETNLFEQVNAGACGLKLHEDWGTTTPSTINSCLNVADTLDVQV 245

Query: 240 AIHT 243
 IHT
 Sbjct: 246 CIHT 249

tr Q9L644 UreC [ureC] [Prochlorococcus marinus] 569 AA
 Q9L644_PROMA align

Score = 268 bits (686), Expect = 6e-71
 Identities = 136/244 (55%), Positives = 167/244 (67%), Gaps = 3/244 (1%)

Query: 1 KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYE 59
 K Y YGPT GDRVRL DT+LI+EVE D TTYG+E+KFGGGK IRDGM Q+ +
 Sbjct: 8 KTYAQTYGPTKGDRVRLADTELIIEVEKDFTTYGDEVKFGGGKVIRDGMGQSQVTREDGA 67

Query: 60 LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAEG 119
 +D V+TNALIVD+ +QD + N+ +G +TE +A EG
 Sbjct: 68 VDTVITNALIVDWWGIVKADVGLKDGKIYEIGKAGNPDIQDNI--NIIIGSSTEVIAGEG 125

Query: 120 LIVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRLA 179
 I+TAG IDTHIHFI PQQI TA ASGVTTM+GGGTGPA GTNATT TPG ++ M+++
 Sbjct: 126 HILTAGSIDTHIHFI CPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMIS 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
 AE + +NLGF KGN S E +L +Q+ AGA G K+HEDWG+TP+ I+ CLNVAD DVQV
 Sbjct: 186 AEAFPVNLGFFGKGNSSNETNLFEQVNAGACGLKLHEDWGTTTPSTINSCLNVADTLDVQV 245

Query: 240 AIHT 243
 IHT
 Sbjct: 246 CIHT 249

sp P77837 Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) 569
 URE1_BACSU [ureC] AA
 [Bacillus subtilis] align

Score = 268 bits (685), Expect = 7e-71
 Identities = 131/245 (53%), Positives = 169/245 (68%), Gaps = 2/245 (0%)

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 +EY ++GPTTGD++RLGDTDL +EVE D T YGEE+ FGGGKTIRDGM Q + +
 Sbjct: 6 EEYAELEFGPTTGDKIRLGDTDLWIEVEKDFTVYGEEMIFGGGKTIRDGMGQNGRITGKD 65

Query: 60 -LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNLCVGPATEALAAE 118
 LDIV+TN +++DYT + DQVD ++ +G TE ++ E
 Sbjct: 66 ALDLVITNVVLLDYTGIVKADVGVKGRIVGVKSGNPDIMDGVDPHVMVIGAGTEVISGE 125

Query: 119 GLIVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMRL 178
 G I+TAGG+DTHIHFI PQQ+ A +SGVTT++GGGTGPA G+ ATT T G + ML
 Sbjct: 126 GKILTAGGVDTHIHFI CPQQMEVALSSGVTTLLGGGTGPATGSKATTCTSGAWYMARMLE 185

Query: 179 AAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQ 238
 AEEE+ +N+GFL KGN S + L +Q+EAGAIG K+HEDWG+TP+AI C+ V DE D+Q
 Sbjct: 186 AAEEFPINVGFLGKGNASDKAPLIEQVEAGAIGLKLHEDWGTTPSAIKTCMEVVDEADIQ 245

Query: 239 VAIHT 243
 VAIHT
 Sbjct: 246 VAIHT 250

tr Q6I6I8 Urease beta subunit (Fragment) [ureB] [Campylobacter 454
 Q6I6I8_CAMLA lari] AA
align

Score = 268 bits (685), Expect = 7e-71
 Identities = 140/238 (58%), Positives = 165/238 (68%), Gaps = 1/238 (0%)

Query: 6 MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
 MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S + DLVLT
 Sbjct: 1 MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66 NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVNDCVGPATEALAAEGLIVTAG 125
 NALIVDYT +QDGV+ +L +G +T+ + AEGGLIVTAG
 Sbjct: 60 NALIVDYTGIIYKADIGIKNGYIVGIGKAGNPDIQDGVNPSLVIGTSTDIIGAEGGLIVTAG 119

Query: 126 GIDTHIHFIISPQQIPTAFASGVTTMIGGGTGPDAGTNATTITPGRANLKSMLRAAEEYAM 185
 GIDTHIHFIISP QI A SGVTTMIG GP++GTNATT T G ++ SML+A + Y M
 Sbjct: 120 GIDTHIHFIPTQIECALYSGVTTMIGARIGPSEGTNATTCTSGAYHIHSMKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
 N GFL KGN S + +L++QI AGA G KIHEDWG+T + I LN+ADE D+QVAI T
 Sbjct: 180 NFGFLGKGNSSKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIQT 237

Database: EXPASY/UniProtKB
 Posted date: Jun 6, 2005 10:37 AM
 Number of letters in database: 640,866,274
 Number of sequences in database: 1,974,938

Lambda	K	H
0.315	0.135	0.393

Gapped

Lambda	K	H
0.267	0.0410	0.140

Matrix: BLOSUM62
 Gap Penalties: Existence: 11, Extension: 1
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 length of database: 640,866,274
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 effective length of query: 121
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 effective search space: 48390784398
 effective search space used: 48390784398

T: 11
A: 40
X1: 16 (7.3 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 42 (22.0 bits)
S2: 72 (32.3 bits)

Wallclock time: 28 seconds

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entry Q9RGP5

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[\[Features\]](#) [\[Sequence\]](#) [\[Tools\]](#)

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	Q9RGP5_HELFE
Primary accession number	Q9RGP5
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 26, March 2004
Name and origin of the protein	
Protein name	Urease [Fragment]
Synonyms	None
Gene name	Name: ureB
From	Helicobacter felis [TaxID: 214]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

- [1] NUCLEOTIDE SEQUENCE.
STRAIN=ATCC51211;
 Weir S.C., Stock F., Gill V.J., Fischer S.H.;
 Submitted (DEC-1998) to the EMBL/GenBank/DDBJ databases.

Comments

None

Cross-references

EMBL	AF116580; AAF21996.1; -; Genomic_DNA.	[EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P14917; 1E9Y. [HSSP ENTRY / PDB]	
SMR	Q9RGP5; 1-243.	
	GO:0016787; Molecular function: hydrolase activity (<i>inferred from electronic annotation</i>).	
	GO:0016151; Molecular function: nickel ion binding (<i>inferred from electronic annotation</i>).	
GO	GO:0009039; Molecular function: urease activity (<i>inferred from electronic annotation</i>).	
	GO:0006807; Biological process: nitrogen compound metabolism (<i>inferred from electronic annotation</i>).	

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view.

InterPro IPR006680; Amidohydro_1.
 IPR005848; Urease_alpha.
 IPR011612; Urease_alpha_N.
 Graphical view of domain structure.

Pfam PF01979; Amidohydro_1; 1.
 PF00449; Urease_alpha; 1.
 Pfam graphical view of domain structure.

PROSITE PS01120; UREASE_1; 1.

HOGENOM [Family / Alignment / Tree]

ProtoMap Q9RGP5.

PRESAGE Q9RGP5.

ModBase Q9RGP5.

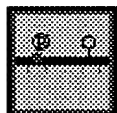
SWISS-2DPAGE Get region on 2D PAGE.

UniRef View cluster of proteins with at least 50% / 90% identity.

Keywords

None

Features



Feature table viewer

Key	From	To	Length	Description
NON_TER	1	1		
NON_TER	243	243		

Sequence information

Length: **243 AA** [This is the length of the partial sequence]

Molecular weight: **25607 Da** [This is the MW of the partial sequence]

CRC64: **A9E2764C95B4BC62** [This is a checksum on the sequence]

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<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>
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EEYAMNLGFL	AKGNVSYEPS	LRDQIEAGAI	GFKIHEDWGS	TPAAIHHCLN	VADEYDVQVA


IHT

Q9RGP5 in FASTA
format

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or at NCBI (USA)



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Compute pI/Mw, PeptideMass, PeptideCutter,
Dotlet (Java)



ScanProsite, MotifScan



Submit a homology modeling request to SWISS-
MODEL



NPSA Sequence analysis
tools



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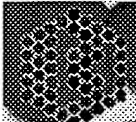
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EBI Dbfetch

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AC  AF116580;
XX
SV  AF116580.1
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DT  15-APR-2005 (Rel. 83, Last updated, Version 2)
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KW  .
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OS  Helicobacter felis
OC  Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacteriales;
OC  Helicobacteraceae; Helicobacter.
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RP  1-732
RA  Weir S.C., Stock F., Gill V.J., Fischer S.H.;
RT  "Helicobacter species differentiation based on DNA sequence variation in a
RT  region of the ureB gene";
RL  Unpublished.
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RP  1-732
RA  Weir S.C., Stock F., Gill V.J., Fischer S.H.;
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RL  Submitted (28-DEC-1998) to the EMBL/GenBank/DDBJ databases.
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☐ CDD ☒ MGC ☐ HPRD ☐ STS

☐ 1: [X69080](#). Reports *H.felis* ureA and ...[gi:396160] [Links](#)

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 ORGANISM *Helicobacter felis*
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 Helicobacteraceae; Helicobacter.
 REFERENCE 1 (bases 1 to 2619)
 AUTHORS Ferrero,R.L. and Labigne,A.
 TITLE Cloning, expression and sequencing of *Helicobacter felis* urease
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 JOURNAL Mol. Microbiol. 9 (2), 323-333 (1993)
 PUBMED 8412683
 REFERENCE 2 (bases 1 to 2619)
 AUTHORS Ferrero,R.L.
 TITLE Direct Submission
 JOURNAL Submitted (02-NOV-1992) R.L. Ferrero, Institut Pasteur, Unite des
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ORIGIN

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Feb 9 2005 14:31:10

view.

InterPro

IPR006680; Amidohydro_1.
 IPR005848; Urease_alpha.
 IPR011612; Urease_alpha_N.
 Graphical view of domain structure.

Pfam

PF01979; Amidohydro_1; 1.
 PF00449; Urease_alpha; 1.
 Pfam graphical view of domain structure.

PROSITE

PS01120; UREASE_1; 1.

HOGENOM

[Family / Alignment / Tree]

ProtoMap

Q9RGP5.

PRESAGE

Q9RGP5.

ModBase

Q9RGP5.

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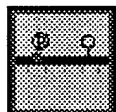
UniRef

View cluster of proteins with at least 50% / 90% identity.

Keywords

None

Features



Feature table viewer

Key	From	To	Length	Description
NON_TER	1	1		
NON_TER	243	243		

Sequence information

Length: **243 AA** [This is the length of the partial sequence]
 Molecular weight: **25607 Da** [This is the MW of the partial sequence]

CRC64: **A9E2764C95B4BC62** [This is a checksum on the sequence]

<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>50</u>	<u>60</u>
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<u>70</u>	<u>80</u>	<u>90</u>	<u>100</u>	<u>110</u>	<u>120</u>
DLVLTNALIV	DYTGIIYKADI	GIKDGKIAGI	GKAGNKDMQD	GVDNNLCVGP	ATEALAAEGL
<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>
IVTAGGIDTH	IHFISPQQIP	TAFASGVTTM	IGGGTGPDAG	TNATTITPGR	ANLKSMRLAA
<u>190</u>	<u>200</u>	<u>210</u>	<u>220</u>	<u>230</u>	<u>240</u>
EEYAMNLGFL	AKGNVSYEPS	LRDQIEAGAI	GFKIHEDWGS	TPAAIHHCLN	VADEYDVQVA


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Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	Q9RGP5_HELFE
Primary accession number	Q9RGP5
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 26, March 2004
Name and origin of the protein	
Protein name	Urease [Fragment]
Synonyms	None
Gene name	Name: ureB
From	Helicobacter felis [TaxID: 214]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

- [1] NUCLEOTIDE SEQUENCE.
STRAIN=ATCC51211;
 Weir S.C., Stock F., Gill V.J., Fischer S.H.;
 Submitted (DEC-1998) to the EMBL/GenBank/DDBJ databases.

Comments

None

Cross-references

EMBL	AF116580; AAF21996.1; -; Genomic_DNA.	[EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P14917; 1E9Y. [HSSP ENTRY / PDB]	
SMR	Q9RGP5; 1-243.	
	GO:0016787; Molecular function: hydrolase activity (<i>inferred from electronic annotation</i>).	
	GO:0016151; Molecular function: nickel ion binding (<i>inferred from electronic annotation</i>).	
GO	GO:0009039; Molecular function: urease activity (<i>inferred from electronic annotation</i>).	
	GO:0006807; Biological process: nitrogen compound metabolism (<i>inferred from electronic annotation</i>).	

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END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 29 of 29 returned.

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Terms	Documents
(felis and urease).ti,ab,clm.	29

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US006406703B1

(12) United States Patent
Doidge et al.**(10) Patent No.: US 6,406,703 B1**
(45) Date of Patent: *Jun. 18, 2002**(54) TREATMENT OF *H. PYLORI* ASSOCIATED GASTRODUODENAL DISEASE****(75) Inventors:** Christopher Vincent Doidge, Box Hill; Adrian Lee, Lanc Cove, both of (AU)**(73) Assignees:** CSL Limited, Victoria (AU); The University of New South Wales, New South Wales (AU)**(*) Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 09/610,937**(22) Filed:** Jul. 5, 2000**Related U.S. Application Data****(63)** Continuation of application No. 09/182,062, filed on Oct. 29, 1998, now Pat. No. 6,129,923, which is a continuation of application No. 08/464,854, filed as application No. PCT/AU94/00416 on Jul. 25, 1994, now Pat. No. 5,871,749.**(30) Foreign Application Priority Data**Jul. 27, 1993 (AU) 0157/93
Feb. 14, 1994 (AU) 3828/94**(51) Int. Cl.⁷** A61K 39/02; A61K 39/16; A61K 9/64; A61K 39/85; C07K 1/00**(52) U.S. Cl.** 424/234.1; 424/203.1; 424/460; 424/461; 424/450; 424/457; 424/500; 424/194.1; 424/501; 424/502; 530/350**(58) Field of Search** 530/350; 424/234.1; 424/194.1, 500, 501, 502, 460, 461, 450, 457, 203.1; 435/7.21**(56) References Cited****U.S. PATENT DOCUMENTS**5,370,872 A 12/1994 Cryz et al. 424/194.1
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Primary Examiner—Lynette R. F. Smith
Assistant Examiner—Ginny Allen Portner
(74) Attorney, Agent, or Firm—Foley & Lardner**(57) ABSTRACT**

The application discloses a method for the treatment of Helicobacter infection in a mammalian host, which comprises administration to said infected host of an immunologically effective amount of one or more Helicobacter antigen(s), optionally in association with a mucosal adjuvant.

41 Claims, No Drawings

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TREATMENT OF *H. PYLORI* ASSOCIATED GASTRODUODENAL DISEASE

This application is a continuation of application Ser. No. 09/182,062, filed Oct. 29, 1998, now U.S. Pat. No. 6,129, 923 which is a continuation of application Ser. No. 08/464, 854 filed Aug. 18, 1995, now U.S. Pat. No. 5,871,749, which is a national stage of PCT/AU94/00416 FILED Jul. 25, 1994.

FIELD OF THE INVENTION

This invention relates to the treatment of gastroduodenal disease associated with *Helicobacter pylori* infection and in particular it relates to the use of active immunisation as a treatment for *H. pylori*—associated gastroduodenal disease.

BACKGROUND OF THE INVENTION

The bacterium, *Helicobacter pylori*, is now well established as a major gastroduodenal pathogen, and more than 50% of the world population is infected with this organism which causes gastritis of varying severity. While no symptoms are apparent in a great proportion of infected persons, in a significant number of *H. pylori* infected persons overt disease may result. The majority (95%) of duodenal ulcers are associated with *H. pylori* infection; a causal role is shown by treatment studies which indicate that if the organisms can be eradicated at the time of ulcer healing then the ulcers do not recur—in contrast to 80% recurrence rate at one year in those who remain infected with the organisms. Furthermore, up to 80% of gastric ulcers are thought to be *H. pylori* associated (Blaser, 1992).

There is now increasing evidence of the harmful consequence of long term *H. pylori* infection. In countries such as China, Colombia and Japan the bacterium is picked up very early in life, and in these persons the gastritis slowly progresses until after 30–40 years of continual infection, severe gastric atrophy appears. Gastric atrophy is well documented as being the precursor lesion for gastric cancer, although the actual cancer that develops in an atrophied stomach is dependent on a myriad of other factors including diet. However, all the evidence to date would suggest that the cancer would not develop if it was possible to remove the *H. pylori* infection at an early age before the atrophy had developed (Parsonnet et al., 1991).

There is no laboratory animal model of *H. pylori* infection that can be used for large scale assessment of new anti-*H. pylori* therapies. However, a *Helicobacter felis* mouse model of gastric *Helicobacter* infection has been developed that has proved extremely useful in the screening of the potential of new antimicrobial therapeutic regimens. *H. felis* is a spiral shaped bacterium that is very closely related to *H. pylori*. This bacterium colonises the stomach of mice in a very similar way to *H. pylori* in the human, i.e. the main ecological niche is gastric mucus and the localisation of colonisation is antral dominant. In germfree mice, *H. felis* infection induces a gastritis that is very similar to the human *H. pylori* infection with a chronic inflammation accompanied by polymorphonuclear leucocyte infiltration. Infection with each organism results in the induction of a similar raised immune response against *H. pylori* and *H. felis* respectively (Lee et al., 1990).

The *H. felis* mouse model has proved to be very predictive of the efficacy of anti-*H. pylori* agents in humans. Thus, monotherapy with agents with high in vitro activity such as erythromycin show no significant in vivo effect against *H. felis* in mice, just as erythromycin has no anti-*H. pylori*

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effect in humans despite high antimicrobial effects in vitro. In contrast, the triple therapy regimens of a bismuth compound, metronidazole, and tetracycline or amoxycillin lead to a very high eradication rate in *H. felis* infected mice (Dick-Hegedus and Lee, 1991). Such triple therapies are the most successful human anti-*H. pylori* regimens, and at the present time are recommended as the first choice for anti-*H. pylori* therapy. However, established *Helicobacter* infections are difficult to treat, and current chemotherapeutic regimens remain suboptimal due to problems with efficacy, toxicity, drug resistance and reinfection (O'Connor, 1992).

Active immunisation of already infected patients has not been proven efficacious for any clinically manifest human infectious disease (Burke, 1992). Given that *H. pylori* infections persist for long periods, if not the life of the infected individual, despite the presence of a vigorous immune response that includes a high level of circulating IgG antibody in the serum and the demonstration of local specific IgA antibody in the gastric mucosa, it has been considered that active immunisation was unlikely to be effective in therapy (Goodwin, 1993). Indeed, Czinn et al. (1993) in proposing that oral vaccination may be a feasible approach for the prevention of *H. pylori* infection in humans (based on an evaluation of an oral immunisation protocol in the *H. felis* mouse model), suggested that once infection is established neither antibody nor antibiotics are very effective at eradication.

Varga et al. (1992) have reported that a *H. pylori* vaccine prepared from organisms derived from a patient, and injected parenterally into that patient, resulted in an allergic reaction and failure to eradicate the organism.

Surprisingly, it has now been discovered for the first time that there is indeed a therapeutic potential for active immunisation against gastric *Helicobacter* infection. Furthermore, it has been discovered that oral administration of *H. pylori* antigen, with a suitable mucosal adjuvant, does not result in allergic or hypersensitivity symptoms, but results in suppression or eradication of the infecting organisms from the gastric mucosa.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a method for the treatment of *Helicobacter* infection in a mammalian host, which comprises the oral administration to said infected host of an immunologically effective amount of one or more *Helicobacter* antigen(s), optionally in association with a mucosal adjuvant.

In another aspect, there is provided a vaccine composition for the treatment of *Helicobacter* infection in a mammalian host, which comprises an immunologically effective amount of one or more *Helicobacter* antigen(s), optionally in association with a mucosal adjuvant.

In yet another aspect, the present invention provides the use of a vaccine composition comprising an immunologically effective amount of one or more *Helicobacter* antigen(s), optionally in association with a mucosal adjuvant, in the treatment of *Helicobacter* infection in a mammalian host.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

By use of the term "immunologically effective amount" herein, it is meant that the administration of that amount to an individual infected host, either in a single dose or as part

of a series, is effective for treatment of *Helicobacter* infection. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the assessment of the medical situation, and other relevant factors. It is expected that the amount will all in a relatively broad range that can be determined through routine trials.

DETAILED DESCRIPTION OF THE INVENTION

The *Helicobacter* antigen(s) used in accordance with the present invention may be *H. felis* antigen(s), or more preferably *H. pylori* antigen(s). In a particularly preferred aspect of the present invention, a vaccine composition comprising *H. pylori* antigen(s) in association with a mucosal adjuvant is used the treatment of *H. pylori* infection in a human patient.

Preferably, the *Helicobacter* antigen(s) comprise a bacterial sonicate, and in particular a *H. pylori* sonicate. More preferably, the *Helicobacter* antigen(s) used in accordance with the present invention comprise inactivated whole bacterial cells of *H. pylori*.

Alternatively, the *Helicobacter* antigen(s) used in accordance with the present invention may comprise one or more individual antigens, particularly one or more *H. pylori* antigens such as *H. pylori* urease, or *H. pylori* cytotoxin (CT), Cytotoxin Associated Immunodominant (CAI) antigen or heat shock protein (hsp) as disclosed by way of example in International Patent Publication No. WO 93/18150.

One mucosal adjuvant which is optionally, and preferably, administered with the *Helicobacter* antigen(s) to the infected host is cholera toxin. Another preferred mucosal adjuvant which may be administered with the *Helicobacter* antigen(s) is *E. coli* heat labile toxin (*E. coli* HLT). Mucosal adjuvants other than cholera toxin and *E. coli* HLT which may be used in accordance with the present invention include non-toxic derivatives of cholera toxin, such as the B sub-unit (CTB), chemically modified cholera toxin, or related proteins produced by modification of the cholera toxin amino acid sequence. Each of these molecules with mucosal adjuvant or delivery properties may be added to, or conjugated with, the *Helicobacter* antigen(s). Other compounds with mucosal adjuvant or delivery activity may be used, such as: bile; polycations such as DEAE-dextran and polyornithine; detergents such as sodium dodecyl benzene sulphate; lipid-conjugated materials; antibiotics such as streptomycin; vitamin A; and other compounds that alter the structural or functional integrity of mucosal surfaces. Other mucosally active compounds include derivatives of microbial structures such as MDP; acridine and cimetidine.

Helicobacter antigen(s) may be delivered in accordance with this invention in ISCOMS (immune stimulating complexes), ISCOMS containing CTB, liposomes or encapsulated in compounds such as acrylates or poly(DL-lactide-co-glycoside) to form microspheres of a size suited to adsorption by M cells. Alternatively, micro or nanoparticles may be covalently attached to molecules such as vitamin B12 which have specific gut receptors. Antigen(s) may also be incorporated into oily emulsions and delivered orally. Ail extensive though not exhaustive list of adjuvants can be found in Cox and Coulter, 1992.

Other adjuvants, as well as conventional pharmaceutically acceptable carriers, excipients, buffers or diluents, may also

be included in the therapeutic vaccine composition of this invention. The vaccine composition may, for example, be formulated in enteric coated gelatine capsules including sodium bicarbonate buffers together with the *Helicobacter* antigen(s) and mucosal adjuvant.

Generally, a vaccine composition in accordance with the present invention will comprise an immunologically effective amount of *Helicobacter* antigen(s), and optionally a mucosal adjuvant, in conjunction with one or more conventional pharmaceutically acceptable carriers and/or diluents. As used herein "pharmaceutically acceptable carriers and/or diluents" include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art and is described by way of example in *Remington's Pharmaceutical Sciences*, 18th Edition, 1990, Mack Publishing Company, Pennsylvania, U.S.A..

The pharmaceutical composition of this invention may be orally administered directly to the mammalian host, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatine capsule, or it may be compressed into tablets, or it may be incorporated directly with the solid or liquid food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of active component in the compositions and preparations may of course be varied and is such that a suitable dosage will be obtained to be immunologically effective.

Solid oral dosage units such as tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active component, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed.

The vaccine composition of the invention is administered orally in amounts readily determined by persons of ordinary skill in this art. Thus, for adults a suitable dosage would be in the range of 10 μ g to 10 g, for example 50 μ g to 3 g. Similar dosage ranges would be applicable for children.

As noted above, a suitable mucosal adjuvant is cholera toxin. The amount of mucosal adjuvant employed depends on the type of mucosal adjuvant used. For example, when the mucosal adjuvant is cholera toxin, it is suitably used in an amount of 10 nanogram to 50 μ g, for example 01 μ g to 10 μ g. When the mucosal adjuvant is *E. coli* heat labile toxin, suitable amounts are 1 μ g to 1 mg, for example 5 μ g to 50 μ g.

In work leading to the present invention, active immunisation of mice previously infected with *H. felis*, with oral

doses of cholera toxin or *E. coli* HLT adjuvant and a whole cell *H. felis* or *H. pylori* sonicate, result in the clearance of *H. felis* from the gastric mucosa. It is therefore anticipated that active immunisation of infected humans with oral doses of a mucosal adjuvant with *H. pylori* antigen(s) will result in the clearance of *H. pylori* from the gastric mucosa. Based on previous studies with this model using anti-*H. pylori* agents, it is considered that this is the first evidence of the therapeutic potential of active immunisation with *H. pylori* vaccines, and indicates that a vaccine composition for the therapy of human *H. pylori*—associated gastroduodenal disease is a preparation of Helicobacter antigen(s), optionally and preferably combined with a mucosal adjuvant.

It will be apparent to persons skilled in the field that effective treatment of *Helicobacter pylori* infection in humans with an oral vaccine composition of Helicobacter antigen(s) which will eradicate or suppress the infection will provide a significant therapeutic benefit via the suppression or elimination of gastritis, prevention of peptic ulcer relapse and reduction in the harmful sequelae of *Helicobacter pylori* infection including peptic ulceration and gastric cancer.

The present invention is further illustrated in the following, non-limiting Examples.

EXAMPLE 1

One hundred and sixty female SPF mice from the Animal Breeding Unit of the University of New South Wales, Australia, were infected with four oral doses of 10^9 – 10^{10} living *Helicobacter felis* (ATCC culture 49179) given two days apart.

Bacteria were grown in plastic Petri dishes on Blood Agar Base No. 2, 3.8% w/v (Oxoid, Basingstoke, U.K.) with 7% v/v whole horse blood (Oxoid), containing amphotericin B (Fungizone, Squibb, Princeton, N.J., USA) 2.5 mg/l; trimethoprim (Sigma, St. Louis, Mo., USA), 10 mg/l. Plates were incubated in a microaerophilic humid atmosphere (Oxoid, BR56) at 37° C. for 48 hours.

Sonicates were prepared by growth of the organisms, as described above, followed by harvesting of the organisms in 0.1 molar phosphate buffered saline (PBS). The cells were washed, collected by centrifugation, washed once in PBS, and resuspended in fresh PBS. The cells were then sonicated at the rate of one minute per ml of cell suspension (50% duty cycle) using a B-30 Branson Cell Disrupter. The sonicate was stored at -20° C.

On days 28, 42, 44 and 47 after administration of the last infecting dose of *H. felis*, 20 of the mice were given orally 0.2 ml of a suspension containing 10 µg of cholera toxin (Sigma C 3012) and a sonicate of *H. felis* containing 1 mg protein (BIO-RAD DC protein assay).

Samples of antral mucosa were tested for infection using a rapid microtitre urease test as described previously (Lee et al., 1990). This test has been validated as being highly predictive of *H. felis* gastric infection. Groups of 40 mice (20 vaccinates and 20 controls) were euthanased at intervals of 1 week, 1 month, 2 months and 3 months after the last dose of vaccine.

The results are shown in Table 1.

These results show that treatment of *H. felis* infected mice with an oral vaccine comprised of Helicobacter antigens and a mucosal adjuvant, results in cure of the infection in a significant proportion of mice. This effect is evident 1 week after cessation of therapy, and continues for at least 3 months, demonstrating that the mice have been cured of their infection.

TABLE 1

Immunisation	Proportion of <i>H. felis</i> infected mice			
	1 week	1 month	2 months	3 months
Nil	19/19	20/20	18/19	13/19
Sonicate plus CT	2/20 P < 0.0001*	3/20 P < 0.0001	6/20 P < 0.05	1/17 P < 0.0001

*Fisher's exact test (two tailed).

EXAMPLE 2

One hundred female BALB/c mice from the Animal Breeding Unit of the University of New South Wales, Australia, were infected with 3 oral doses of 10^8 living *Helicobacter felis* (ATCC culture 49179) given 2 days apart, i.e. days 1, 3 and 5.

Bacteria were grown in plastic Petri dishes on Blood Agar Base No. 2, 3.8% w/v (Oxoid, Basingstoke, U.K.) with 7% v/v whole horse blood, (Oxoid), containing amphotericin B (Fungizone, Squibb, Princeton, N.J., USA) 2.5 mg/l; trimethoprim (Sigma, St. Louis, Mo., USA), 10 mg/l. Plates were incubated in a microaerophilic humid atmosphere (Oxoid, BR56) at 37° C. for 48 hours.

Sonicates were prepared by growth of the organisms, as described above, followed by harvesting of the organisms in 0.1 molar phosphate buffered saline (PBS). The cells were washed collected by centrifugation, washed once in PBS, and resuspended in fresh PBS. The cells were then sonicated at the rate of one per minute per ml of cell suspension (50% duty cycle) using a B-30 Branson Cell Disrupter. The sonicate was stored at -20° C.

On days 21, 35, 37, and 40 after administration of the last infecting dose of *H. felis*, 20 mice were each given orally 0.2 ml of a solution containing 10 µg of cholera toxin (Sigma C 3012), 20 mice were each given orally 0.2 ml of a suspension containing 10 µg of cholera toxin and a sonicate of *H. felis* containing 1 mg protein (BIO-RAD DC protein assay), 20 mice were each given orally 0.2 ml of a suspension containing a sonicate of *H. felis* containing 1 mg protein, 20 mice were each given orally 0.2 ml of a suspension containing 10 µg of cholera toxin and a sonicate of *H. pylori* (strain 921023) containing 1 mg protein, and 20 mice were not orally vaccinated.

One week after the final immunising dose all the mice were euthanased. Samples of antral mucosa were tested for infection using a rapid microtitre urease test as described previously (Lee et al., 1990). This test has been validated as being highly predictive of *H. felis* gastric infection.

The results are shown in Table 2.

These results show that oral administration of Helicobacter antigens derived from either *H. felis*, or *H. pylori* along with a mucosal adjuvant, will cure a significant portion of *H. felis* infected mice.

TABLE 2

Vaccine	Number of animals infected	Significance
Nil	16/20	
CT alone	15/20	N.S.
<i>H. felis</i> sonicate alone	12/20	N.S.
<i>H. felis</i> sonicate plus CT	8/19	P < 0.05*
<i>H. pylori</i> sonicate plus CT	4/20	P < 0.001

*Fisher's exact test (two tailed)

EXAMPLE 3

One hundred female SPF mice from the Animal Breeding Unit of the University of New South Wales, Australia, were

infected with 4 oral doses of 10^9 – 10^{10} living *Helicobacter felis* (ATCC culture 49179) given 2 days apart. 20 female SPF mice were left uninfected, as negative controls.

Bacteria were grown in plastic Petri dishes on Blood Agar Base No. 2, 3.8% w/v (Oxoid, Basingstoke, UK) with 7% v/v whole horse blood (Oxoid), containing amphotericin B (Fungizone, Squibb, Princeton, N.J., USA) 2.5 mg/l; trimethoprim (Sigma, St. Louis, Mo., USA), 10 mg/l. Plates were incubated in a microaerophilic humid atmosphere (Oxoid, BR56) at 37° C. for 48 hours.

Sonicates were prepared by growth of the organisms, as described above, followed by harvesting of the organisms in 0.1 molar phosphate buffered saline (PBS). The cells were washed, collected by centrifugation, washed once in PBS, and resuspended in fresh PBS. The cells were then sonicated at the rate of one per minute per ml of cell suspension (50% duty cycle) using a B-30 Branson Cell Disrupter. The sonicate was stored at –20° C.

Starting between 6 weeks and 9 weeks after their last infecting dose of *H. felis*, 20 mice were each given orally 0.2 ml of a solution containing 25 µg of *E. coli* heat labile toxin (HLT) (Sigma E 8015), 20 mice were each given orally 0.2 ml of a suspension containing 25 µg of HLT and a sonicate of *H. pylori* containing 1 mg protein (BIO-RAD DC protein assay), 20 mice were each given orally 0.2 ml of a suspension containing a sonicate of *H. pylori* containing 1 mg protein, and 40 mice were not orally vaccinated.

Each group received three further doses 15, 17 and 20 days after their initial dose.

Four weeks after the final immunising dose all the mice were euthanased. Samples of antral mucosa were tested for infection using a rapid microtitre urease test as described previously (Lee et al., 1990). This test has been validated as being highly predictive of *H. felis* gastric infection.

The results are shown in Table 3.

They show that oral administration of *Helicobacter* antigens derived from *H. pylori* along with a mucosal adjuvant *E. coli* heat labile toxin, will cure a significant portion of *H. felis* infected mice.

TABLE 3

Treatment Group	Proportion of <i>H. felis</i> infected mice
Uninfected, unvaccinated	0/20
Infected, unvaccinated	40/40
Infected, Hp antigen alone	20/20
Infected, <i>E. coli</i> HLT alone	20/20
Infected, Hp antigen & HLT	6/19*

*P < 0.0001 (Fisher's exact test, two tailed).

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What is claimed is:

1. A method of producing a composition, the method comprising combining:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) a mucosal adjuvant;
- (c) an antibiotic; and
- (d) a pharmaceutically acceptable carrier or diluent;

thereby producing a composition that is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

2. The method of claim 1, wherein said one or more *Helicobacter* antigens comprise one or more *H. pylori* antigens.

3. The method of claim 1, wherein said one or more *Helicobacter* antigens comprise one or more *H. felis* antigens.

4. The method of claim 1, wherein said one or more *Helicobacter* antigens are provided in a sonicate of *Helicobacter* cells.

5. The method of claim 1, wherein said adjuvant is cholera toxin, a non-toxic derivative of cholera toxin, or *E. coli* heat labile toxin.

6. The method of claim 1, wherein the mucosal adjuvant has mucosal delivery activity.

7. The method of claim 1, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

8. A composition comprising:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant; and
- (d) an immune stimulating complex (ISCOM);

wherein the composition is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

9. A composition comprising:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant; and
- (d) a liposome;

wherein the composition is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

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10. A composition comprising:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant; and
- (d) a microsphere;

wherein the composition is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

11. A composition comprising:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant; and
- (d) an oily emulsion;

wherein the composition is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

12. A composition comprising:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant; and
- (d) an enteric coated gelatin capsule;

wherein the composition is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

13. A composition comprising:

- (a) an immunologically effective amount of one or more *Helicobacter* antigens;
- (b) an antibiotic; and
- (c) a pharmaceutically acceptable carrier or diluent,

wherein the composition is effective for eradicating or suppressing a pre-existing *Helicobacter* infection in a mammalian host when administered to the host.

14. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising administration of the composition of claim 8 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

15. The method of claim 14, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

16. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising oral administration of the composition of claim 8 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

17. The method of claim 16, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

18. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising administration of the composition of claim 9 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

19. The method of claim herein said one or more *Helicobacter* antigens are selected from the group consisting of

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H. pylori urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

20. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising oral administration of the composition of claim 9 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

21. The method of claim 20, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

22. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising administration of the composition of claim 10 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

23. The method of claim 22, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

24. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising oral administration of the composition of claim 10 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

25. The method of claim 24, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

26. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising administration of the composition of claim 11 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

27. The method of claim 26, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

28. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising oral administration of the composition of claim 11 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

29. The method of claim 28, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

30. A method for the treatment of a pre-existing *Helicobacter* infection in a mammalian host, the method comprising administration of the composition of claim 12 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

31. The method of claim 30, wherein said one or more *Helicobacter* antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

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32. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 12 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

33. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

34. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 13 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

35. The method of claim 32, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

36. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 13 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

37. The method of claim 36, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

38. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method compris-

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ing administration to a mucosal surface of the infected host of a composition comprising:

- (a) an immunologically effective amount of one or more Helicobacter antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant and
- (d) a pharmaceutically acceptable carrier or diluent;

wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

39. The method of claim 38, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

40. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration to the infected host of a composition comprising:

- (a) an immunologically effective amount of one or more Helicobacter antigens;
- (b) an antibiotic;
- (c) a mucosal adjuvant and
- (d) a pharmaceutically acceptable carrier or diluent;

wherein the administration of the composition eradicates or suppresses the preexisting infection in the host.

41. The method of claim 40, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

* * * * *

DOCUMENT-IDENTIFIER: US 6406703 B1

TITLE: Treatment of H. pylori associated gastroduodenal disease

CLAIMS:

3. The method of claim 1, wherein said one or more Helicobacter antigens comprise one or more H. felis antigens.
7. The method of claim 1, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
15. The method of claim 14, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
17. The method of claim 16, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
19. The method or claim herein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
21. The method of claim 20, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
23. The method of claim 22, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
25. The method of claim 24, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
27. The method of claim 26, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
29. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
31. The method of claim 30, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori i cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.
33. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group

consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

35. The method of claim 32, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

37. The method of claim 36, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

39. The method of claim 38, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

41. The method of claim 40, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

DOCUMENT-IDENTIFIER: US 6290962 B1

**** See image for Certificate of Correction ****

TITLE: Urease-based vaccine and treatment for helicobacter infection

Abstract Text (1):

Method of eliciting in a mammalian host a protective immune response to Helicobacter infection and treatment of Helicobacter infection by administering to the host an immunogenically effective amount of a Helicobacter urease or urease subunits as antigen. Vaccine compositions are also provided.

CLAIMS:

1. A method of treating Helicobacter infection in a mammal, said method comprising administering to a mucosal surface of said mammal a therapeutically effective amount of a purified polypeptide comprising an A subunit of a naturally occurring Helicobacter urease.
3. The method of claim 1, wherein said Helicobacter urease is Helicobacter pylori urease.
21. A method of treating Helicobacter infection in a mammal, said method comprising administering to a mucosal surface of said mammal a therapeutically effective amount of a purified polypeptide comprising a B subunit of a naturally occurring Helicobacter urease.
23. The method of claim 21, wherein said Helicobacter urease is Helicobacter pylori urease.
41. A vaccine composition consisting essentially of a polypeptide comprising an A subunit of a naturally occurring Helicobacter urease, and a mucosal adjuvant.
42. The vaccine composition of claim 41, wherein said Helicobacter urease is Helicobacter pylori urease.
43. The vaccine composition of claim 41, wherein said Helicobacter urease is Helicobacter felis urease.
54. A vaccine composition comprising a purified polypeptide comprising an A subunit of a naturally occurring Helicobacter urease, and a polypeptide of the labile toxin of Escherichia coli.
56. A vaccine composition consisting essentially of a polypeptide comprising a B subunit of a naturally occurring Helicobacter urease, and a mucosal adjuvant.
57. The vaccine composition of claim 56, wherein said Helicobacter urease is Helicobacter pylori urease.
58. The vaccine composition of claim 56, wherein said Helicobacter urease is Helicobacter felis urease.
69. A vaccine composition comprising a purified polypeptide comprising a B subunit of a naturally occurring Helicobacter urease, and a polypeptide of the labile toxin of Escherichia coli.

US-PAT-NO: 6290962

DOCUMENT-IDENTIFIER: US 6290962 B1

**** See image for Certificate of Correction ****TITLE: Urease-based vaccine and treatment for helicobacter infection

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Michetti; Pierre	Lausanne			CH
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Blum; Andre	Romammotier			CH
Davin; Catherine	Nyon			CH
Haas; Rainier	Tubingen			CH
Kraehenbuhl; Jean-Pierre	Rivat			CH
Saraga; Emilia	Lausanne			CH

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
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APPL-NO: 08/ 200346 [PALM]

DATE FILED: February 23, 1994

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/085,938, filed Jul. 6, 1993 now U.S. Pat. No. 5,972,336, which is a continuation-in-part application of U.S. application Ser. No. 07/970,996, filed Nov. 3, 1992, now abandoned the whole of which applications (including drawings) are hereby incorporated by reference.

INT-CL: [07] A61 K 39/00, A61 K 38/46, A61 K 31/70, A61 K 39/385

US-CL-ISSUED: 424/185.1; 424/234.1, 424/184.1, 424/192.1, 424/193.1, 424/197.1, 424/261.1, 424/280.1, 424/278.1, 424/94.6, 424/282.1, 424/203.1, 514/41, 514/234.5

US-CL-CURRENT: 424/185.1; 424/184.1, 424/192.1, 424/193.1, 424/197.11, 424/203.1, 424/234.1, 424/261.1, 424/278.1, 424/280.1, 424/282.1, 424/94.6, 514/234.5, 514/41

FIELD-OF-SEARCH: 424/234.1, 424/192.1, 424/193.1, 424/197.11, 424/261.1, 424/278.1, 424/280.1, 424/185.1, 424/184.1, 424/94.6, 424/282.1, 424/203.1, 514/41, 514/234.5

PRIOR-ART-DISCLOSED:

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<input type="checkbox"/> 5403924	April 1995	Cover et al.	536/23.1
<input type="checkbox"/> 5538729	July 1996	Czinn et al.	
<input type="checkbox"/> 5578302	November 1996	Brassart et al.	
<input type="checkbox"/> 5733740	March 1998	Cover et al.	435/7.32
<input type="checkbox"/> 5859219	January 1999	Cover et al.	536/22.1
<input type="checkbox"/> 5871749	February 1999	Doidge et al.	424/234.1
<input type="checkbox"/> 5972336	October 1999	Michetti et al.	
<input type="checkbox"/> 5985631	November 1999	Soman et al.	
<input type="checkbox"/> 6060241	May 2000	Corthesy-Theulaz	
<input type="checkbox"/> 6096521	August 2000	Haas et al.	

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WO 94/09823	November 1994	WO	
WO 95/03824	February 1995	WO	

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ART-UNIT: 165

PRIMARY-EXAMINER: Mannfield; Nita

ATTY-AGENT-FIRM: Clark & Elbing LLP

ABSTRACT:

Method of eliciting in a mammalian host a protective immune response to *Helicobacter* infection and treatment of *Helicobacter* infection by administering to the host an immunogenically effective amount of a *Helicobacter* urease or urease subunits as antigen. Vaccine compositions are also provided.

72 Claims, 10 Drawing figures

DOCUMENT-IDENTIFIER: US 6258359 B1

**** See image for Certificate of Correction ****

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NO: 22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NO: 20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NO: 20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NO: 22-26);

CLAIMS:

4. A composition comprising purified monoclonal antibodies directed against the following peptides:

a) at least one urease polypeptide of *Helicobacter felis* or *Helicobacter pylori* selected from the group consisting of UreA, UreB, UreE, UreF, UreG, UreH, UreI, and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment; and

b) at least one polypeptide of *Helicobacter felis* or *Helicobacter pylori* selected from the group consisting of HspA (SEQ ID NO: 29), HspB (SEQ ID NO: 30), and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment.

5. The composition of claim 4, wherein said urease polypeptide is UreA of *Helicobacter pylori*.

6. The composition of claim 4, wherein said urease polypeptide is UreB of *Helicobacter pylori*.

7. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of *Helicobacter pylori*.

8. The composition of claim 4, wherein said urease polypeptide is UreA of *Helicobacter felis*.

9. The composition of claim 4, wherein said urease polypeptide is UreB of *Helicobacter felis*.

10. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of *Helicobacter felis*.

US-PAT-NO: 6258359

DOCUMENT-IDENTIFIER: US 6258359 B1

**** See image for Certificate of Correction ****

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Suerbaum; Sebastian	Veitshochheim			DE
Ferrero; Richard L.	Paris			FR
Thiberge; Jean-Michel	Plaisir			FR

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Institut Pasteur	Paris			FR	03

APPL-NO: 08/ 466248 [PALM]

DATE FILED: June 6, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional application of application Ser. No. 08/447,177, filed May 19, 1995, now abandoned, which is a continuation-in-part of application Ser. No. 08/432,697, filed May 2, 1995, which is a continuation-in-part of International Application PCT/EP94/01625, filed May 19, 1994, which is based on International Application PCT/EP93/03259, filed Nov. 19, 1993, and European Application No. 93 401 309.5, filed May 19, 1993. Applicants claim the benefits of the International filing dates and priority of the European filing date. The entire disclosure of each of these applications is relied upon and incorporated by reference herein.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	93 401 309	May 19, 1993
WO	PCT/EP93/03259	November 19, 1993

INT-CL: [07] A61 K 39/395, A61 K 39/40, C07 K 1/00, C07 K 16/00

US-CL-ISSUED: 424/141.1; 424/150.1, 424/163.1, 424/164.1, 530/350, 530/388.1, 530/388.2, 530/388.4

US-CL-CURRENT: 424/141.1; 424/150.1, 424/163.1, 424/164.1, 530/350, 530/388.1, 530/388.2, 530/388.4

FIELD-OF-SEARCH: 424/234.1, 424/141.1, 424/150.1, 424/163.1, 424/164.1, 514/2, 530/350, 530/388.1, 530/388.2, 530/388.4

PRIOR-ART-DISCLOSED:

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Search Selected

Search ALL

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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ART-UNIT: 165

PRIMARY-EXAMINER: Navarro; Albert

ATTY-AGENT-FIRM: Finnegan, Henderson, Farabow, Garrett and Dunner

ABSTRACT:

There is provided an immunogenic composition capable of inducing protective antibodies against *Helicobacter* infection characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NO: 22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NO: 20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NO: 20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NO: 22-26);
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from *Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

20 Claims, 51 Drawing figures



US006258359B1

(12) **United States Patent**
Labigne et al.

(10) **Patent No.:** US 6,258,359 B1
(45) **Date of Patent:** Jul. 10, 2001

(54) **IMMUNOGENIC COMPOSITIONS AGAINST
HELICOBACTER INFECTION,
POLYPEPTIDES FOR USE IN THE
COMPOSITIONS, AND NUCLEIC ACID
SEQUENCES ENCODING SAID
POLYPEPTIDES**

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(DE); Richard L. Ferrero, Paris;
Jean-Michel Thiberge, Plaisir, both of
(FR)

(73) **Assignee:** Institut Pasteur, Paris (FR)

(*) **Notice:** Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** 08/466,248

(22) **Filed:** Jun. 6, 1995

Related U.S. Application Data

(60) Division of application No. 08/447,177, filed on May 19,
1995, now abandoned, which is a continuation-in-part of
application No. 08/432,697, filed on May 2, 1995, which is
a continuation-in-part of application No. PCT/EP94/01625,
filed on May 19, 1994.

(30) Foreign Application Priority Data

May 19, 1993 (EP) 93 401 309
Nov. 19, 1993 (WO) PCT/EP93/03259

(51) **Int. Cl.⁷** A61K 39/395; A61K 39/40;
C07K 1/00; C07K 16/00

(52) **U.S. Cl.** 424/141.1; 424/150.1;
424/163.1; 424/164.1; 530/350; 530/388.1;
530/388.2; 530/388.4

(58) **Field of Search** 424/234.1, 141.1,
424/150.1, 163.1, 164.1; 514/2; 530/350,
388.1, 388.2, 388.4

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tic Assays for Helicobacter Pylori with Established Clinical
and Histopathologic Findings", *Gastroenterology*, vol. 100,
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(List continued on next page.)

Primary Examiner—Albert Navarro

(74) *Attorney, Agent, or Firm*—Finnegan, Henderson,
Farabow, Garrett and Dunner

(57) ABSTRACT

There is provided an immunogenic composition capable of
inducing protective antibodies against Helicobacter infec-
tion characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide
from *Helicobacter pylori* (SEQ ID NO: 22,26), or a
fragment thereof, said fragment being recognized by
antibodies reacting with *Helicobacter felis urease*
(SEQ ID NO: 20-21), and/or at least one sub-unit of a
urease structural polypeptide from *Helicobacter felis*
(SEQ ID NO: 20-21), or a fragment thereof, said
fragment being recognized by antibodies reacting with
Helicobacter pylori urease (SEQ ID NO: 22-26);
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from
Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immuno-
genic compositions is also provided.

20 Claims, 36 Drawing Sheets

DOCUMENT-IDENTIFIER: US 6248330 B1

**** See image for Certificate of Correction ****

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis urease*, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori urease*;

CLAIMS:

10. Proteinaceous material comprising a fusion protein, wherein the fusion protein comprises at least one *Helicobacter* HspA or a fragment thereof as defined in any one of claims 6-9 and at least one polypeptide selected from the group consisting of

a *Helicobacter pylori urease* structural polypeptide or fragment thereof, wherein said fragment is recognized by antibodies to H. *felis urease*, and

a *Helicobacter felis urease* structural polypeptide or immunogenic fragment thereof.

11. An immunogenic composition, which induces antibodies against *Helicobacter* infection, comprising at least one sub-unit of a purified, synthetic, or recombinant *Helicobacter felis urease* structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, and a heat shock protein (Hsp) from *Helicobacter* or a fragment thereof, wherein the Hsp protein is HspA or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), and wherein said fragment has at least 6 amino acids and is immunogenic.

16. An immunogenic composition, capable of inducing antibodies against *Helicobacter* infection, comprising at least one sub-unit of a purified, synthetic, or recombinant *Helicobacter felis urease* structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, further comprising at least one heat shock protein (Hsp) from *Helicobacter*, wherein the Hsp protein is HspA, HspB, or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), or a fragment thereof, wherein said fragment has at least 6 amino acids and is capable of generating antibodies.



US006248330B1

(12) **United States Patent**
Labigne et al.

(10) **Patent No.:** US 6,248,330 B1
(45) **Date of Patent:** *Jun. 19, 2001

(54) **IMMUNOGENIC COMPOSITIONS AGAINST
HELICOBACTER INFECTION,
POLYPEPTIDES FOR USE IN THE
COMPOSITIONS, AND NUCLEIC ACID
SEQUENCES ENCODING SAID
POLYPEPTIDES**

(75) **Inventors:** Agnes Labigne, Bures sur Yvette (FR);
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Richard L. Ferrero, Paris;
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(FR)

(73) **Assignee:** Institut Pasteur, Paris (FR)

(*) **Notice:** Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-
claimer.

(21) **Appl. No.:** 08/432,697

(22) **Filed:** May 2, 1995

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/EP94/01625,
filed on May 19, 1994.

(30) Foreign Application Priority Data

May 19, 1993 (EP) 93401309
May 19, 1994 (WO) PCT/EP94/03259

(51) **Int. Cl.⁷** A61K 39/00

(52) **U.S. Cl.** 424/192.1; 424/234.1;
424/184.1; 435/6; 435/69.1

(58) **Field of Search** 424/234.1, 184.1,
424/203.1, 192.1; 435/6, 7.21

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English).*

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Primary Examiner—Christopher L. Chin

Assistant Examiner—Ginny Allen Portner

(74) *Attorney, Agent, or Firm*—Finnegan, Henderson,
Farabow, Garrett & Dunner

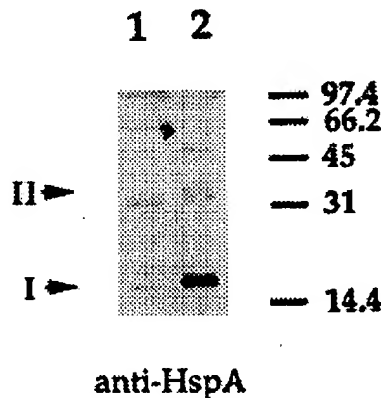
(57) ABSTRACT

There is provided an immunogenic composition capable of
inducing protective antibodies against *Helicobacter* infec-
tion characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide
from *Helicobacter pylori*, or a fragment thereof, said
fragment being recognized by antibodies reacting with
Helicobacter felis urease, and/or at least one sub-unit of
a urease structural polypeptide from *Helicobacter felis*,
or a fragment thereof, said fragment being recognized
by antibodies reacting with *Helicobacter pylori* urease;
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from
Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immuno-
genic compositions is also provided.

16 Claims, 36 Drawing Sheets



TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22-26);

1. An immunogenic composition, capable of inducing antibodies against *Helicobacter* infection, comprising:

ii) at least one heat shock protein encoded by the Hsp A gene of *Helicobacter pylori* or *Helicobacter felis* or immunogenic fragment thereof, comprising at least 6 consecutive amino acids,

2. An immunogenic composition comprising an immunizing amount of a mixture of *Helicobacter pylori* or *Helicobacter felis* antigens, wherein said mixture consists essentially of UreB and HspA of *H. pylori* or *H. felis* substantially free of other *H. pylori* or *H. felis* proteins.

10. A pharmaceutical composition for use in a vaccine against *Helicobacter pylori* or *Helicobacter felis*, comprising the immunogenic composition according to claim 1 or claim 2, in combination with a pharmaceutically acceptable carrier.



US005843460A

United States Patent [19]

Labigne et al.

[11] **Patent Number:** 5,843,460[45] **Date of Patent:** Dec. 1, 1998

[54] **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

[75] **Inventors:** Agnes Labigne, Bures S/Yvette, France; Sebastin Suerbaum, Bochum, Germany; Richard L. Ferrero, Paris; Jean-Michel Thiberge, Plaisir, both of France

[73] **Assignees:** Institut Pasteur; Institut National de la Sante et de la Recherche Medicale, both of Paris, France

[21] **Appl. No.:** 467,822

[22] **Filed:** Jun. 6, 1995

Related U.S. Application Data

[63] Continuation of Ser. No. 447,177, May 19, 1995, which is a continuation-in-part of Ser. No. 432,697, May 2, 1995.

[30] Foreign Application Priority Data

May 19, 1993 [EP] European Pat. Off. 93 401 309
Nov. 19, 1993 [WO] WIPO PCT/EP93/03259

[51] **Int. Cl.⁶** A61K 39/02

[52] **U.S. Cl.** 424/234.1; 435/7.32; 435/6; 435/7.9; 514/234.5; 514/41

[58] **Field of Search** 435/7.32, 4, 6, 435/7.9; 514/234.5, 41; 424/234.1

[56] References Cited**FOREIGN PATENT DOCUMENTS**

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WOA9109049 6/1991 WIPO .
WOA9307273 4/1993 WIPO .
WOA9316723 9/1993 WIPO .
WOA9318150 9/1993 WIPO .
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Primary Examiner—James C. Housel

Assistant Examiner—Ginny Allen Portner

Attorney, Agent, or Firm—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

[57] ABSTRACT

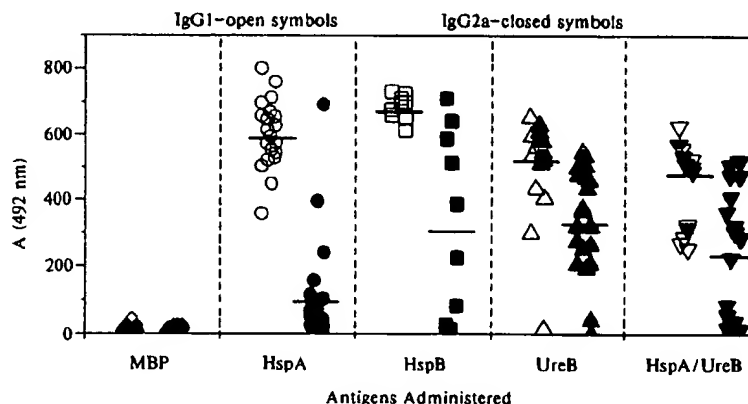
There is provided an immunogenic composition capable of inducing protective antibodies against *Helicobacter infection* characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22-26);

ii) and/or, a heat shock protein (Hsp), or chaperonin, from *Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

10 Claims, 36 Drawing Sheets



PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 39/106, C07K 16/12, A61K 39/40		A1	(11) International Publication Number: WO 96/34624
			(43) International Publication Date: 7 November 1996 (07.11.96)
(21) International Application Number: PCT/EP96/01834		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 2 May 1996 (02.05.96)		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(30) Priority Data: 432,697 2 May 1995 (02.05.95) US 447,177 19 May 1995 (19.05.95) US			
(71) Applicants (for all designated States except US): INSTITUT PASTEUR [FR/FR]; 25-28, rue du Dr.-Roux, F-75724 Paris Cédex 15 (FR). INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cédex 13 (FR).			
(72) Inventors; and (75) Inventors/Applicants (for US only): LABIGNE, Agnès [FR/FR]; 47, avenue Beauséjour, F-91440 Bures-sur-Yvette (FR). SUERBAUM, Sébastien [DE/DE]; Kanalstrasse 6, D-44787 Bochum (DE). FERRERO, Richard, L. [IT/FR]; 60, avenue des Gobelins, F-75013 Paris (FR). THIBERGE, Jean-Michel [FR/FR]; 15, rue de la Ferronnerie, F-78370 Plaisir (FR).			
(74) Agents: GUTMANN, Ernest et al.; Ernest Gutmann-Yves Plasseraud S.A., 3, rue Chauveau-Lagarde, F-75008 Paris (FR).			
(54) Title: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES			
(57) Abstract <p>There is provided an immunogenic composition capable of inducing protective antibodies against <i>Helicobacter</i> infection characterized in that it comprises: i) at least one sub-unit of a urease structural polypeptide from <i>Helicobacter pylori</i>, or a fragment thereof, said fragment being recognized by antibodies reacting with <i>Helicobacter felis</i> urease, and/or at least one sub-unit of a urease structural polypeptide from <i>Helicobacter felis</i>, or a fragment thereof, said fragment being recognized by antibodies reacting with <i>Helicobacter pylori</i> urease; ii) and/or, a heat shock protein (Hsp), or chaperonin, from <i>Helicobacter</i>, or a fragment of said protein. The preparation, by recombinant means, of such immunogenic composition is also provided.</p>			

L2: Entry 21 of 29

File: EPAB

Nov 7, 1996

DOCUMENT-IDENTIFIER: WO 9634624 A1

TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION,
POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES
ENCODING SAID POLYPEPTIDES

Abstract Text (1):

There is provided an immunogenic composition capable of inducing protective antibodies against Helicobacter infection characterized in that it comprises: i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori, or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease, and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis, or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease; ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein. The preparation, by recombinant means, of such immunogenic composition is also provided.

DOCUMENT-IDENTIFIER: WO 9514093 A1

TITLE: IMMUNOGENIC COMPOSITIONS AGAINST *HELICOBACTER* INFECTION,
POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES
ENCODING SAID POLYPEPTIDES

Abstract Text (1):

CHG DATE=19990617 STATUS=O>The invention relates to an immunogenic composition, capable of inducing protective antibodies against *Helicobacter* infection, characterised in that it comprises: i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognised by antibodies reacting with *Helicobacter felis urease*, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognised by antibodies reacting with *Helicobacter pylori urease*; ii) optionally, a urease-associated Heat Shock protein (HSP), or chaperonin, from *Helicobacter*, or a fragment of said protein.

**TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION,
POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES
ENCODING SAID POLYPEPTIDES**

CHG DATE=19990617 STATUS=O>The invention relates to an immunogenic composition, capable of inducing protective antibodies against *Helicobacter* infection, characterised in that it comprises: i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognised by antibodies reacting with *Helicobacter felis urease*, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognised by antibodies reacting with *Helicobacter pylori urease*; ii) and/or, a Heat Shock protein (HSP), or chaperonin, from *Helicobacter*, or a fragment of said protein. The invention also relates to the preparation, by recombinant means, of such immunogenic compositions.

Mark Patent	Mark Range	Mark Section	◀◀	24	of 29	▶▶	◀◀	All Sections	▶▶	Print
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15. Document ID: US 6005090 A

L2: Entry 15 of 29

File: USPT

Dec 21, 1999

DOCUMENT-IDENTIFIER: US 6005090 A

TITLE: Treatment and prevention of helicobacter infection

Abstract Text (1):

An antigenic preparation for use in the treatment or prevention of Helicobacter infection in a mammalian host, comprises the catalase enzyme of Helicobacter bacteria, particularly the catalase enzyme of H. pylori or H. felis, or an immunogenic fragment thereof.

CLAIMS:

8. A vaccine composition for use in the treatment or prevention of Helicobacter infection in a mammalian host, consisting essentially of an immunologically effective amount of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

9. A vaccine composition for use in the treatment or prevention of Helicobacter infection in a mammalian host, consisting essentially of an immunologically effective amount of Helicobacter pylori catalase, together with Helicobacter urease and Helicobacter lipopolysaccharide, and further together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

10. A method for the treatment or prevention of Helicobacter infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation consisting essentially of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

11. A method for the treatment or prevention of Helicobacter infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation consisting essentially of Helicobacter pylori catalase, together with Helicobacter urease and Helicobacter lipopolysaccharide, and further together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

12. A method of producing a vaccine, comprising the step of bringing an antigenic preparation into a form suitable for administration to a mammal, wherein said preparation consists essentially of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, and wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

13. Method of producing a vaccine, comprising the step of bringing an antigenic preparation into a form suitable for administration to a mammal, wherein said preparation consists essentially of Helicobacter pylori catalase, and Helicobacter urease and Helicobacter lipopolysaccharide, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KNOW	Drawn
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☐ 16. Document ID: US 5871749 A

L2: Entry 16 of 29

File: USPT

Feb 16, 1999

DOCUMENT-IDENTIFIER: US 5871749 A

TITLE: Therapeutic treatment of H. pylori associated gastroduodenal disease

CLAIMS:

3. A method according to claim 1, wherein said one or more Helicobacter antigens comprise one or more H. felis antigens.

12. A method according to claim 10, wherein said one or more Helicobacter antigens comprise one or more H. felis antigens.

19. A method according to claim 11, wherein said one or more Helicobacter antigens is selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

20. A method according to claim 10, wherein said one or more Helicobacter antigens is selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	ROME	Drawings
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☐ 17. Document ID: US 5843460 A

L2: Entry 17 of 29

File: USPT

Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843460 A

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):

i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease (SEQ ID NOS:22-26);

CLAIMS:

1. An immunogenic composition, capable of inducing antibodies against Helicobacter infection, comprising:

i) at least one urease structural polypeptide encoded by the UreB gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof

comprising at least six consecutive amino acids; and

ii) at least one heat shock protein encoded by the Hsp A gene of *Helicobacter pylori* or *Helicobacter felis* or immunogenic fragment thereof, comprising at least 6 consecutive amino acids,

said composition being substantially free of other *Helicobacter pylori* or *Helicobacter felis* proteins.

2. An immunogenic composition comprising an immunizing amount of a mixture of *Helicobacter pylori* or *Helicobacter felis* antigens, wherein said mixture consists essentially of UreB and HspA of *H. pylori* or *H. felis* substantially free of other *H. pylori* or *H. felis* proteins.

6. The immunogenic composition according to claim 1 or claim 2, wherein said composition produces an immunogenic effect when administered to a mammal, wherein the immunogenic effect is substantially the same as the immunogenic effect produced in the mammal when a total cell extract of *Helicobacter pylori* or *Helicobacter felis* is administered to said mammal.

10. A pharmaceutical composition for use in a vaccine against *Helicobacter pylori* or *Helicobacter felis*, comprising the immunogenic composition according to claim 1 or claim 2, in combination with a pharmaceutically acceptable carrier.

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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18. Document ID: JP 2004337170 A

L2: Entry 18 of 29

File: JPAB

Dec 2, 2004

PUB-NO: JP02004337170A

DOCUMENT-IDENTIFIER: JP 2004337170 A

TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: December 2, 2004

INVENTOR-INFORMATION:

NAME

COUNTRY

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SUERBAUM, SEBASTIEN

FERRERO, RICHARD

THIBERGE, JEAN-MICHEL

INT-CL (IPC): C12 N 15/09; A61 K 38/00; A61 K 39/00; A61 K 39/39; A61 K 39/395; A61 P 1/04; A61 P 35/00; A61 P 37/02; C07 K 14/205; C07 K 16/12; C07 K 16/40; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 Q 1/68; C12 P 21/08

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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19. Document ID: JP 2002355054 A

L2: Entry 19 of 29

File: JPAB

Dec 10, 2002

PUB-NO: JP02002355054A
DOCUMENT-IDENTIFIER: JP 2002355054 A
TITLE: HELICOBACTER FELIS VACCINE

PUBN-DATE: December 10, 2002

INVENTOR-INFORMATION:

NAME

COUNTRY

KUSTERS, JOHANNES GERARDUS

CATTOLI, GIOVANNI

INT-CL (IPC): C12 N 15/09; A61 K 38/00; A61 K 39/106; A61 K 39/118; A61 K 39/12;
A61 K 39/175; A61 K 39/23; A61 K 39/235 ; A61 K 39/39; A61 K 39/395; A61 P 1/04;
A61 P 31/04; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 Q
1/68; G01 N 33/15; G01 N 33/50; G01 N 33/53; G01 N 33/566; G01 N 33/569

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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☐ 20. Document ID: EP 1176192 A2

L2: Entry 20 of 29

File: EPAB

Jan 30, 2002

PUB-NO: EP001176192A2
DOCUMENT-IDENTIFIER: EP 1176192 A2
TITLE: Helicobacter felis vaccine

PUBN-DATE: January 30, 2002

INVENTOR-INFORMATION:

NAME

COUNTRY

KUSTERS, JOHANNES GERARDUS

NL

CATTOLI, GIOVANNI

IT

INT-CL (IPC): C12 N 9/80; C07 K 14/205; C07 K 16/12; C12 Q 1/68; A61 K 39/106; A61
K 48/00; G01 N 33/53; G01 N 33/68
EUR-CL (EPC): C07K014/205; C12N009/80

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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☐ 21. Document ID: WO 9634624 A1

L2: Entry 21 of 29

File: EPAB

Nov 7, 1996

PUB-NO: WO009634624A1
DOCUMENT-IDENTIFIER: WO 9634624 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR
USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: November 7, 1996

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	FR
SUERBAUM, SEBASTIAN	DE
FERRERO, RICHARD L	FR
THIBERGE, JEAN-MICHEL	FR

INT-CL (IPC): A61 K 39/106; C07 K 16/12; A61 K 39/40

EUR-CL (EPC): A61K039/106; C07K014/205, C07K016/12

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. De
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☐ 22. Document ID: WO 9514093 A1

L2: Entry 22 of 29

File: EPAB

May 26, 1995

PUB-NO: WO009514093A1

DOCUMENT-IDENTIFIER: WO 9514093 A1

TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: May 26, 1995

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	
SUERBAUM, SEBASTIEN	
FERRERO, RICHARD	

INT-CL (IPC): C12 N 15/31; C12 N 9/80; C12 Q 1/68; C12 P 21/08; A61 K 39/106

EUR-CL (EPC): C07K014/205; C12N009/80, C12Q001/68

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. De
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☐ 23. Document ID: WO 9426901 A1

L2: Entry 23 of 29

File: EPAB

Nov 24, 1994

PUB-NO: WO009426901A1

DOCUMENT-IDENTIFIER: WO 9426901 A1

TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: November 24, 1994

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	FR

SUERBAUM, SEBASTIEN
 FERRERO, RICHARD
 THIBERGE, JEAN-MICHEL

FR
 FR
 FR

INT-CL (IPC): C12N 15/31; C12N 9/80; C12Q 1/68; C12P 21/08; A61K 39/106; G01N 33/577

EUR-CL (EPC): C07K014/205; C12N009/80, C12Q001/68

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw De
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☐ 24. Document ID: ES 2208519 T3, EP 1176192 A2, AU 200154321 A, CA 2351110 A1, JP 2002355054 A, EP 1176192 B1, DE 60100879 E, US 20040005325 A1

L2: Entry 24 of 29

File: DWPI

Jun 16, 2004

DERWENT-ACC-NO: 2002-124384

DERWENT-WEEK: 200442

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TITLE: Novel *Helicobacter felis* urease X and Y subunit polypeptides, useful in the diagnosis of *Helicobacter felis* infections and in the preparation of vaccines

INVENTOR: CATTOLI, G; KUSTERS, J G

PRIORITY-DATA: 2000EP-0202565 (July 17, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>ES 2208519 T3</u>	June 16, 2004		000	C12N009/80
<u>EP 1176192 A2</u>	January 30, 2002	E	076	C12N009/80
<u>AU 200154321 A</u>	January 24, 2002		000	C12N015/52
<u>CA 2351110 A1</u>	January 17, 2002	E	000	C12N015/55
<u>JP 2002355054 A</u>	December 10, 2002		149	C12N015/09
<u>EP 1176192 B1</u>	October 1, 2003	E	000	C12N009/80
<u>DE 60100879 E</u>	November 6, 2003		000	C12N009/80
<u>US 20040005325 A1</u>	January 8, 2004		000	A61K039/00

INT-CL (IPC): A61 K 38/00; A61 K 39/00; A61 K 39/106; A61 K 39/118; A61 K 39/12; A61 K 39/175; A61 K 39/23; A61 K 39/235; A61 K 39/38; A61 K 39/39; A61 K 39/395; A61 K 48/00; A61 P 1/04; A61 P 31/04; C07 K 14/195; C07 K 14/205; C07 K 16/12; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 N 15/09; C12 N 15/52; C12 N 15/55; C12 Q 1/68; G01 N 33/15; G01 N 33/50; G01 N 33/53; G01 N 33/566; G01 N 33/569; G01 N 33/68; C12 N 9/80; C12 Q 1/68; C12 R 1/01; C12 R 1/01

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw De
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☐ 25. Document ID: NZ 519980 A, WO 200152667 A2, AU 200140494 A, EP 1118271 A1, NO 200203434 A, BR 200016973 A, EP 1251747 A2, US 20030049240 A1, JP 2003520038 W, MX 2002007018 A1, ZA 200206546 A

L2: Entry 25 of 29

File: DWPI

Apr 30, 2004

DERWENT-ACC-NO: 2002-280180

DERWENT-WEEK: 200431

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TITLE: Use of lactic acid bacteria strain(s), metabolites or fermented medium, having anti-Helicobacter activity in vitro, in preparation of composition for prophylaxis/treatment of gastric disorders in pets

INVENTOR: BALLEVRE, O; CORTHESEY-THEULAZ, I ; ENSLEN, M Y A ; ENSLEN, A M

PRIORITY-DATA: 2000EP-0200179 (January 18, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>NZ 519980 A</u>	April 30, 2004		000	A23K001/16
<u>WO 200152667 A2</u>	July 26, 2001	E	027	A23K001/00
<u>AU 200140494 A</u>	July 31, 2001		000	A23K001/00
<u>EP 1118271 A1</u>	July 25, 2001	E	000	A23K001/16
<u>NO 200203434 A</u>	August 20, 2002		000	A23K000/00
<u>BR 200016973 A</u>	October 15, 2002		000	A23K001/00
<u>EP 1251747 A2</u>	October 30, 2002	E	000	A23K001/16
<u>US 20030049240 A1</u>	March 13, 2003		000	A61K045/00
<u>JP 2003520038 W</u>	July 2, 2003		033	A23K001/00
<u>MX 2002007018 A1</u>	January 1, 2003		000	A23K001/00
<u>ZA 200206546 A</u>	March 31, 2004		036	A23K000/00

INT-CL (IPC): A23 K 0/00; A23 K 1/00; A23 K 1/16; A23 K 1/18; A61 K 35/74; A61 K 45/00; A61 P 1/04; A61 P 31/04

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Draw Da
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☐ 26. Document ID: WO 200003730 A1, AU 9884643 A

L2: Entry 26 of 29

File: DWPI

Jan 27, 2000

DERWENT-ACC-NO: 2000-171201

DERWENT-WEEK: 200029

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TITLE: Use of Lactobacillus-derived urease, as a vaccine for preventing or treating Helicobacter infection and conditions including gastric ulcer, gastritis and gastric cancer caused by the infection

INVENTOR: PARK, J B

PRIORITY-DATA: 1998WO-KR00216 (July 16, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>WO 200003730 A1</u>	January 27, 2000	E	038	A61K039/07
<u>AU 9884643 A</u>	February 7, 2000		000	A61K039/07

INT-CL (IPC): A61 K 39/07; C07 K 14/335

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. D.
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☐ 27. Document ID: WO 9911284 A1, AU 9888779 A

L2: Entry 27 of 29

File: DWPI

Mar 11, 1999

DERWENT-ACC-NO: 1999-204981

DERWENT-WEEK: 199931

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TITLE: Vaccine containing lactobacilli expressing urease peptide - for treating gastrointestinal disorders

INVENTOR: TABAQCHALI, S; WILKS, M

PRIORITY-DATA: 1997GB-0018616 (September 2, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>WO 9911284 A1</u>	March 11, 1999	E	023	A61K039/02
<u>AU 9888779 A</u>	March 22, 1999		000	A61K039/02

INT-CL (IPC): A61 K 38/43; A61 K 39/02; C12 N 15/74

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. D.
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☐ 28. Document ID: WO 9634624 A1, AU 9656934 A

L2: Entry 28 of 29

File: DWPI

Nov 7, 1996

DERWENT-ACC-NO: 1996-505900

DERWENT-WEEK: 199904

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TITLE: New immunogenic compsn. contg. UreB and HspA antigens of Helicobacter - for treatment and prevention of esp. H pylori infection, also new antibodies specific for these antigens.

INVENTOR: FERRERO, R L; LABIGNE, A ; SUERBAUM, S ; THIBERGE, J

PRIORITY-DATA: 1995US-0447177 (May 19, 1995), 1995US-0432697 (May 19, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>WO 9634624 A1</u>	November 7, 1996	E	184	A61K039/106
<u>AU 9656934 A</u>	November 21, 1996		000	A61K039/106

INT-CL (IPC): A61 K 39/106; A61 K 39/40; C07 K 16/12

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. D.
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29. Document ID: JP 2004337170 A, WO 9426901 A1, AU 9469290 A, EP 703981 A1, JP 08510120 W, AU 689779 B, AU 9875081 A, SG 52480 A1, US 5843460 A, AU 724584 B, US 6248330 B1, US 6258359 B1

L2: Entry 29 of 29

File: DWPI

Dec 2, 2004

DERWENT-ACC-NO: 1995-006797

DERWENT-WEEK: 200479

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TITLE: DNA from *Helicobacter pylori* and *Helicobacter felis* - used to develop prods. for detection, treatment and prevention of *Helicobacter* infection

INVENTOR: FERRERO, R; LABIGNE, A ; SUERBAUM, S ; THIBERGE, J ; FERRERO, R L

PRIORITY-DATA: 1993WO-EP03259 (November 19, 1993), 1993EP-0401309 (May 19, 1993), 1995JP-0514170 (November 19, 1993)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>JP 2004337170 A</u>	December 2, 2004		063	C12N015/09
<u>WO 9426901 A1</u>	November 24, 1994	E	168	C12N015/31
<u>AU 9469290 A</u>	December 12, 1994		000	C12N015/31
<u>EP 703981 A1</u>	April 3, 1996	E	000	C12N015/31
<u>JP 08510120 W</u>	October 29, 1996		154	C12N015/09
<u>AU 689779 B</u>	April 9, 1998		000	C12N015/31
<u>AU 9875081 A</u>	October 1, 1998		000	A61K039/106
<u>SG 52480 A1</u>	September 28, 1998		000	C12N015/31
<u>US 5843460 A</u>	December 1, 1998		000	A61K039/02
<u>AU 724584 B</u>	September 28, 2000		000	A61K039/106
<u>US 6248330 B1</u>	June 19, 2001		000	A61K039/00
<u>US 6258359 B1</u>	July 10, 2001		000	A61K039/395

INT-CL (IPC): A61 K 38/00; A61 K 39/00; A61 K 39/02; A61 K 39/106; A61 K 39/39; A61 K 39/395; A61 K 39/40; A61 P 1/04; A61 P 35/00; A61 P 37/02; C07 H 21/04; C07 K 1/00; C07 K 14/195; C07 K 14/205; C07 K 16/00; C07 K 16/12; C07 K 16/40; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 N 15/09; C12 N 15/31; C12 P 21/02; C12 P 21/08; C12 Q 1/68; G01 N 33/569; G01 N 33/574; G01 N 33/577

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw Dg
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(felis and urease).ti,ab,clm.	29

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STIC-ILL

196341

From: Portner, Ginny
To: STIC-ILL
Subject: FROM 1802
Date: Tuesday, October 28, 1997 3:59PM

DEVELOPMENT OF AN ANIMAL MODEL TO TEST A *HELICOBACTER-PYLORI* VACCINE
DUNKLEY M L; CRIPPS A W; REINBOTT P W
AUSPHARM INST. MUCOSAL IMMUNOL., P.O. BOX 151, JESMOND, N.S.W. 2299,
AUST.

VITH INTERNATIONAL WORKSHOP ON *CAMPYLOBACTER* *HELICOBACTER* AND RELATED
ORGANISMS, SYDNEY, NEW SOUTH WALES, AUSTRALIA, OCTOBER 7-10, 1991. MICROB
ECOL HEALTH DIS 4 (SPEC. ISSUE). 1991. S148. CODEN: MEHDE

Language: ENGLISH

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Descriptors/Keywords: ABSTRACT RAT GUT FREUND'S COMPLETE ADJUVANT
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ADMINISTRATION ORAL ADMINISTRATION METHOD

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The VIth International Workshop on *Campylobacter helicobacter* and Related Organisms,
Sydney, Australia

VOLUME 4 (S)

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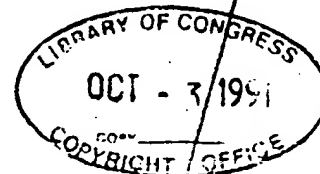
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- C1-2. *Campylobacter* bacteremia - a one year experience
F. Morey, JC Erlich and J. Thurley
- C1-3. *Campylobacter jejuni* septicaemia in a 50 years old mexican
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- C1-4. Two successive outbreaks of *Campylobacter* enteritis in a
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- C1-7. Guillain-Barre syndrome associated with *Campylobacter*
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Nakanishi

C2. IMMUNOLOGY

- C2-1. Adjuvant effect of *Escherichia coli* heat labile enterotoxin on
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Campylobacter antigens
OR Pavlovskis, DM Rollins, FM Rollwagen and RI Walker
- C2-2. Serum immune response to *Campylobacter jejuni* infection in
young children
D Mass Pech, JJ Calva, GM Ruiz-Palacios and Y Lopez-Vidal



1991

DEVELOPMENT OF AN ANIMAL MODEL TO TEST A
HELICOBACTER PYLORI VACCINE

M.L. Dunkley, A.W. Cripps and P.W. Reinbott

Auspharm Institute for Mucosal Immunology
P.O. Box 151, Jesmond NSW 2299

An animal model is being developed to test a vaccine against *H. Pylori* and to investigate the mechanism of the immune response to *H. Pylori* in the gut.

Initially, rats were immunized using several different immunization regimes, viz. intramuscular (IM) immunization with lyophilized *H. Pylori* in Freund's complete adjuvant (FCA), intra-Peyers patch (IPP) immunization with paraformaldehyde-killed *H. Pylori* in FCA and oral immunization with live *H. Pylori*, lyophilized *H. Pylori* or paraformaldehyde-treated *H. Pylori*, all in phosphate buffered saline and with prior administration of sodium bicarbonate.

IM immunization produced a significant enhanced IgG and IgA *H. Pylori* specific antibody response in the serum but had no effect on the salivary antibody response. IPP immunization gave an enhanced serum and saliva IgG and IgA antibody response and the Peyers patch lymphocytes were demonstrated to have a substantial proliferative response to a crude *H. Pylori* antigen preparation in vitro (stimulation index = 64 ± 26) indicating that the gut mucosa is capable of mounting a vigorous immune response against the *H. Pylori* bacteria. Oral immunization however, has been less successful. Oral immunization with live or paraformaldehyde-treated bacteria gave no significant enhancement of the serum or saliva anti-*H. Pylori* antibody. Oral immunization with lyophilized *H. Pylori* gave a small increase in serum antibody response but this was not significant. The enhancement of this response by the addition of adjuvants is under investigation.

H2-4

IMMUNISATION AND GASTRIC COLONISATION WITH *HELICOBACTER FELIS*

Keith Heap and Adrian Lee

University of New South Wales, Sydney, Australia 2033

Introduction

There is accumulating evidence that long term infection with *Helicobacter pylori* is a prerequisite for the development of atrophic gastritis and the subsequent development of gastric cancer in a subset of persons in certain developing countries. Thus, introduction of intervention strategies at an early age may influence the morbidity and mortality of this serious disease. Immunisation would be an attractive option but, given *H. pylori* can survive in the body for tens of years in the presence of a strong immune response, may not be effective. *Helicobacter felis* will colonise the gastric mucosa of SPF mice in large numbers occupying the gastric pits and mucus. Like *H. pylori* in humans this bacterium will remain for the life of the animal. Thus, the *H. felis*-infected mouse would appear to be a good model to test the hypothesis that immunisation can protect against colonisation with gastric helicobacters.

Methods

SPF mice were immunised by intravenous injection of 0.1 ml of a suspension of viable *H. felis* (10^8 / ml) once a week for 5 weeks or infected *per os* over 5 days with three doses of the bacterium. Immune responses of both these groups of animals were measured. A similar group of parenterally immunised animals were challenged with living cultures of *H. felis*. A final group of orally *H. felis*-infected animals was cleared of the organism with triple anti-microbial therapy for 28 days (tetracycline, metronidazole, bismuth subcitrate). These animals and controls that had been given saline instead of triple therapy were then challenged with a living culture of *H. felis*. All challenged animals were assessed for *H. felis* colonisation by rapid urease testing of gastric tissue and histology.

Results

Parenteral immunisation of mice with living cultures of *H. felis* induced a very high level of serum IgG, significant IgM and IgA could be detected in the bile. Serum responses post oral infection were much less and developed slowly. Hyperimmunisation of mice with an intravenous injection of a live culture of *H. felis* had no protective effect on gastric colonisation. In contrast, in mice cleared of infection with *H. felis* by administration of a one month treatment of antibiotics, some effect on rechallenge was seen. Colonisation was significantly delayed, with numbers of animals showing no urease reactivity for up to 10 days after rechallenge with an inoculum of *H. felis* that always gave 100% positivity in normal animals.

Conclusion

Parenteral immunisation with *H. felis* gave absolutely no protection against gastric colonisation. The same is likely to be true for *H. pylori*. However, preliminary experiments show that previous oral infection with living bacteria did appear to have some effect on reinfection. Further experiments are in progress to assess the value of oral immunisation against infection with gastric helicobacters.

Development of a vaccine against *Helicobacter pylori*: a short overview

Rino Rappuoli, Massimo Bugnoli, Paolo Ghiara, Antonello Covacci,
Roberto Olivieri, Zhaoying Xiang and John L. Telford

Aim: Growing evidence that gastric and duodenal disease is caused by *Helicobacter pylori* infection suggests that this disease may be prevented by vaccination. We therefore assessed the possibilities for development of a vaccine.

Method: Survey of published studies.

Present state of development: Development of a vaccine requires identification of the factors responsible for bacterial virulence and disease induction and large-scale production and testing of potential vaccines in animal models. So far several factors involved in bacterial adhesion, colonization and virulence have been identified. Among these, the most promising candidates for vaccine development are the adhesins, the vacuolating cytotoxin and urease. Urease-based vaccines have shown some promising results in protecting mice against *H. felis* infection.

Proposals: The unique features of *H. pylori* infection and disease formation in man suggest that vaccines should be tested in models more relevant to humans, and that the vacuolating cytotoxin and the cytotoxin-associated gene A (*cagA*) should be seriously considered as vaccine candidates. This hypothesis is supported by the recent observation that only the subset of strains that produce the vacuolating cytotoxin and *cagA* are associated with disease.

European Journal of Gastroenterology & Hepatology 1993, 5 (suppl 2):S76-S78

Keywords: Vaccine, cytotoxin-associated gene, vacuolating cytotoxin, *Helicobacter pylori*.

Introduction

The strong correlation between *Helicobacter pylori* infection and the incidence of gastritis, peptic ulcer and gastric cancer has provided conclusive evidence that this infection is a major factor in the genesis of gastric diseases. Vaccination is the classical way of preventing infectious diseases and therefore it is important to study this possibility of controlling *H. pylori* infection.

The development of a vaccine needs four major steps, (1) identification of the factors required for virulence, (2) large-scale production and characterization of the virulence factors, (3) development of appropriate animal models to test the virulence and immunogenicity of the molecules identified, and (4) identification of the type of immunity able to prevent infection and disease.

Identification of virulence factors

Many virulence factors of *H. pylori* have been identified during the last 10 years. These include factors in-

involved in adhesion of the bacteria to eukaryotic cells, toxins and associated proteins, flagella, urease and heat-shock proteins.

Adhesins

Several factors involved in bacterial adhesion have been characterized and some of them have been cloned. These include molecules with weights of 19 600, 20 000 and 63 000 [1-3] that are candidates for inclusion in experimental vaccines, in order to evaluate their ability to induce antibodies able to prevent the bacterial adhesion to the mucosal surface.

Vacuolating cytotoxin and the cytotoxin-associated protein (*CagA*)

The molecule that is believed to be a major factor in *H. pylori* pathogenesis is the vacuolating cytotoxin. This is a protein with a molecular weight of 95 000 that is found in bacterial culture supernatants and on the external surface of the bacteria; it induces vacuolation in HeLa cells or other cell lines of epithelial origin. The cells affected by the toxin form large cytoplasmic vacuoles and die within a few days. Formation of the vacuoles can be inhibited by the antibiotic bafilomycin

From the Immunobiology Research Institute Siena, Siena, Italy.

Requests for reprints to: Dr Rino Rappuoli, Immunobiology Research Institute Siena, Via Fiorentina 1, 53100 Siena, Italy.

A1, a specific inhibitor of eukaryotic vacuolar ATPase, suggesting that vacuole formation requires direct or indirect activation of the vacuolar ATPase enzyme, which pumps protons into the lumen of the vacuole [4].

Recently, it has been shown that only a subset of *H. pylori* isolates are able to induce vacuole formation. These strains produce and secrete in the supernatant the active cytotoxin, a monomer with a molecular weight of 95 000 [5]. Extracts of cytotoxic strains induce the formation of vacuoles not only in HeLa cells, but also in the stomach of experimental animals, suggesting that the cytotoxin is an active disease factor. Cytotoxic *H. pylori* strains also produce a surface-exposed protein of unknown function with a molecular weight of 128 000. This protein, cytotoxin-associated gene A (CagA), varies in size in different isolates [6,7]. The sequence of the protein is well conserved and the difference in size is due to the duplication of a segment of 102 base pairs which encodes for the most hydrophilic portion of the molecule. CagA is the most immunogenic antigen of *H. pylori* and the sera of infected subjects have high levels of antibodies against it. Analysis of sera has shown that 100% of patients with duodenal ulcers and 60% of patients with gastric ulcers have high levels of antibodies against CagA [8]. This observation strongly suggests that disease is caused only by the strains that produce the cytotoxin and CagA, and shows that CagA is a marker for cytotoxic, disease-associated strains. *H. pylori* isolates can therefore be classified into two major categories, the cytotoxin- and CagA-producing strains, which are virulent and induce disease, and the non-cytotoxic strains, which are not associated with disease.

Urease

The urease enzyme is produced by all strains of *H. pylori*, and helps the bacterium to neutralize the acidity of the stomach in order to survive these hostile conditions. The enzyme is also present in other species of *Helicobacter* and is well conserved in the different species. It has two subunits with molecular weights of 64 000 and 30 000 which are assembled in a high-molecular-weight molecule containing two ring-like structures, each composed of six monomers of one of the subunits [9].

Flagella

H. pylori produces polar sheathed flagella, which are essential for the bacterial mobility that allows penetration of the viscous mucous layer coating the gastric mucosa. The flagella consist of two subunits (FlaA and FlaB) with molecular weights of 51 000 and 53 000, respectively. The central core of the flagella is composed of polymerized FlaA subunits surrounded by a membrane-like structure containing proteins and lipopolysaccharide [10].

Heat-shock protein

A protein homologous to the Hsp60 family has been purified from water extracts of *H. pylori*. This anti-

gen is highly homologous to the human homolog and induces antibodies that may react with the self molecule, suggesting a potential role in disease [11].

Production and purification of virulence factors

One of the limiting steps in studying the virulence of *H. pylori* is the difficulty of growing the bacterium on a large scale and of purifying large quantities of antigens. The bacterium grows very slowly under micro-aerophilic conditions in the presence of serum, a medium full of exogenous proteins that makes antigen purification difficult. In order to overcome these problems we have developed a new serum-free medium that allows us to grow the bacterium in synthetic medium containing cyclodextrin [12]. We can then grow the bacterium in fermenters and collect large volumes of supernatant and cell paste. The availability of these materials has allowed, for the first time, large-scale purification of cytotoxin, CagA, urease and Hsp60. These molecules are now being tested *in vitro* and *in vivo* to help determine their contribution to pathogenicity and immunity.

Animal models and vaccine development

H. pylori is found only in primates such as man, macaques, cynomolgous monkeys and baboons. Therefore, these animals are the only relevant models. However, given the limited number of experiments that can be performed in monkeys and man, the high costs and the long time required for the experiments, alternative models are needed. For the moment, the most popular model is *H. felis*, a strain that is easy to work with because it infects mice. However, the results coming from these experiments must be interpreted cautiously because although *H. felis* produces urease, it does not produce the cytotoxin or the CagA antigen. We are at present investigating the possibility of infecting mice with virulent *H. pylori* strains and of mimicking the symptoms of the disease using purified *H. pylori* antigens.

Conclusion

The observation that most gastric pathologies are associated with *H. pylori* infection suggest that vaccination may be successful in preventing infection and disease. To test this hypothesis, several antigens have been identified as candidates for inclusion in experimental vaccines. These are the vacuolating cytotoxin,

CagA, urease, the flagella and the adhesins. These antigens are being tested in animal models.

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WORLD-WIDE

H.pylori associated disease involves host and bacterial factors

Martin Blaser, Scoville Professor of Medicine and Professor of Microbiology and Immunology at the Vanderbilt University School of Medicine, Nashville, USA is a familiar name to those in the field of Campylobacter and Helicobacter research. He recently spoke to HPWW in Sydney and participated in a symposium at the European Digestive Disease Week in Amsterdam in October, speaking on pathogenic mechanisms involved in H.pylori infections.

"H.pylori is a bacterium that the host cannot eliminate", said Blaser. "A critical factor is its ability to live in the lumen of the stomach without needing to invade tissue - it has succeeded where most gastrointestinal bacteria have failed. The host relies on acid, peristalsis, mucus and tight junctions to eliminate organisms from the stomach. H.pylori not only avoids these host defences but initiates disease processes that can result in peptic ulceration and possibly gastric carcinoma."

Blaser proposed that the pathogenic mechanisms be divided into three groups: bacterial virulence factors, autoimmunity and what he terms 'the innocent bystander theory'.

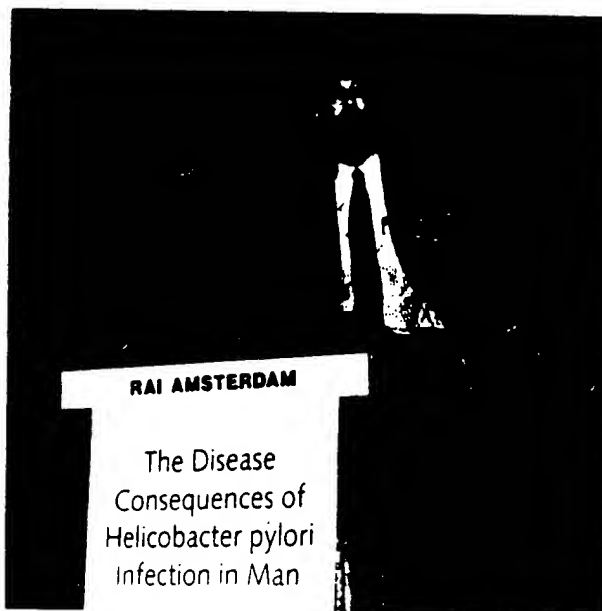
Bacterial virulence factors

While a large number of putative virulence factors have been suggested, increasing attention is focused on the cytotoxin and the urease.

H.pylori is known to produce a toxin that causes cell vacuolation in vitro and this vacuolation is seen in vivo. "We have recently shown that people who are infected produce neutralising IgG antibodies against the cytotoxin - that means that the cytotoxin is produced in vivo", said Blaser.

Work by Blaser's group has shown that antibodies to a 128kDa cytotoxin-associated protein, are seen in 60% of patients with gastritis but in 100% of duodenal ulcer patients. This agrees with earlier work by Figura and colleagues who showed a higher frequency of cytotoxin-producing strains of H.pylori in ulcer patients. "There may be a group of H.pylori strains that put individuals at a higher risk of ulcer", suggested Blaser.

Dr Timothy Cover is currently purifying and characterising the cytotoxin. "Most people find that about 50-60% of strains



Prof. M. Blaser

express the cytotoxin. Once we purify the protein and clone the gene, we will know how many strains have the gene."

Blaser stressed that the cytotoxin and the urease are quite distinct. "It is clear to us that there is a urease effect and a cytotoxin effect and they are independent and additive in vitro. We have looked at urease-minus strains and they had cytotoxin and induced vacuolation. However ammonia augments the effect of the cytotoxin."

"The Helicobacters are the most potent producers of urease known", pointed out Blaser. Recent work has shown that at least nine genes are required for structure and regulation. "I'm impressed by the num-

ber of genes required for the regulation of urease. Obviously this activity is very important to the bacteria as it devotes so much metabolic energy to the production of urease. One of its functions is in acid buffering and facilitating colonisation - we are not yet certain what else it does."

The roles of other H.pylori enzymes such as the mucinase, oxidase, catalase, alkaline phosphatase and phospholipase are not yet clear. "Whether these enzymes are just performing housekeeping functions or are virulence factors for the bacterium, we don't know."

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The innocent bystander

The urease may have a role in pathogenesis quite unrelated to its enzymatic activity" suggested Blaser. The inflammation induced by H. pylori may be perturbing gastric physiology. How does a non-invasive organism cause inflammation?

"Urease is on the surface of H. pylori and there is evidence that it and other surface proteins can be shed from the organism and adsorbed into the lamina propria. We also know that, in vitro, H. pylori supernatants are chemotactic for both monocytes and neutrophils and this effect can be blocked by antibodies to the bacteria or to urease. An N-terminal peptide of the urease appears to be responsible for the chemotactic activity", said Blaser.

"These mononuclear cells are activated, producing superoxide and cytokines such as tissue necrosis factor and interleukin 1. Cytokines are known to stimulate gastrin production and the inflammation per se may be producing the hypergastrinemia seen in H. pylori infection", speculated Blaser.

This recruitment and activation of inflammatory cells may result in a positive feedback cycle, releasing nutrients for H. pylori. Blaser suggested that there are suppressor mechanisms that are trying to down-regulate this inflammation. "It is this inter-

change between suppression and inflammation that ultimately affects gastrin secretion and parietal cell function. This interchange may finally result in a diseased gastrointestinal tract, just as the innocent bystander may be caught in cross-fire between warring parties."

Autoimmunity and heat shock proteins

The Sydney meeting saw the report of a close homology between a surface-exposed, immunodominant H. pylori protein and a human heat-shock protein. All organisms from bacteria to mammalian cells have heat shock proteins, which are normal house-keeping proteins that respond to stress. These proteins are very highly conserved, both structurally and functionally.

"The heat shock protein that we identified with Bruce Dunn is in the chaperonin class of heat shock proteins - chaperonins are involved in the folding and transport of proteins. From our limited sequence data, the heat shock protein resembles the human heat shock protein, hsp 60, expressed on epithelial cells. We know that there is antigenic cross-reaction between H. pylori and a lot of other bacterial heat shock proteins which have been shown to cross-react with hsp 60. We haven't done the direct experiment yet but it is likely that it will cross-react."

Earlier evidence that autoimmunity might play a role came from work by B Rathbone who showed that a monoclonal antibody raised against H. pylori reacted with healthy gastric cells.

What determines outcome?

"We know that different people have different outcomes after infection. Most people have asymptomatic infection, some people develop duodenal ulcer, others gastric ulcer, others atrophic gastritis. The fundamental question is what is the determinant of each of those outcomes", stressed Blaser.

The age at which infection is acquired is almost certain to be one of the determinants, he agreed. In addition, there may be carcinogenic strains or ulcerogenic strains of H. pylori. "The outcome is most likely to be due to a combination of bacterial factors and host factors."

H. pylori affects both the antrum where gastrin is produced and the body where acid is produced. According to Blaser, "There must be a functional balance between the antrum and the body - in some people, the balance is disturbed in one direction or the other. I think it will be very important to characterise this phenomenon because that might determine who gets ulcers and who doesn't."

H. pylori a slow bacterium?

"H. pylori might be the first of a group of organisms considered as slow bacteria", suggested Blaser, drawing an analogy with the so-called slow viruses, such as those responsible for scrapie in sheep and Creutzfeldt-Jacob disease in man. These agents are characterised by a long period of infection with chronic progressive pathology.

"This is a chronic infection as distinct from most bacterial infections which on last a few weeks. The bacteria are active and the host is aware of it, unlike infections such as tuberculosis or chickenpox which become latent and then reactivate. Pathogenecity should be measured not in days but in years." Prof Blaser drew attention to the limitations of experimental models of disease. "Something may be a real virulence factor and cause damage over the course of the infection, that is decades, but the level of the damage is very small so you can't appreciate it in a model that lasts for a couple of days."

"In pathogenesis you always have to remember that there are in vivo events and in vitro events and the two are not necessarily related", cautioned Blaser. "What you see in vitro is not necessarily significant."

6th International Workshop on Campylobacter, Helicobacter and related organisms, October 7-10, 1991, Sydney

More and more Helicobacters

The first Helicobacter (then called Campylobacter pyloridis) was described at the second Campylobacter workshop in Brussels in 1983. These early days were remembered with a presentation to Robin Warren (Australia), the first to notice H. pylori in gastric biopsies. "Twelve years ago, no-one would believe me that the organisms were there," he said.

The number of believers has since steadily increased and many of them attended presentations and workshops on Helicobacter pathogenesis, drug resistance, therapy, immunology, epidemiology, animal models, taxonomy and genetics, in Sydney recently.

The number of Helicobacter species has now reached eight with descriptions at this meeting of H. acinonyx from cheetahs with gastritis (KA Eaton, USA), H. muridarum from the intestinal mucosa of the rodent (A Lee, Australia) and Helicobacter strains from the faeces of terns, gulls, house sparrows and pigs (J Fox, USA).

It appears that what was initially thought to be a homogeneous group confined to the stomach is heterogeneous in terms of habitat, ultrastructure and physiology. Some of these species are urease-negative so urease-positivity can no longer be considered a valid taxonomic marker. The only shared feature seems to be the presence of sheath-

ed flagella. This heterogeneity has stimulated study of the genetics of Helicobacters and the development of genus- and species-specific probes.

Highly variable genome

The Helicobacter genomes are small, extremely variable and subject to natural transformation. Understanding the genetics allows the construction of specific mutants that should help clarify the role of various H. pylori products.

Many of the genetic studies have focused on the urease, which has been shown to have two structural genes, two regulatory genes and five other genes (A Labigne, France).

R Ferrero (France) reported the construction of stable genetically engineered urease mutants of H. pylori, affected either in the structural or the regulatory genes. Urease mutants were also constructed by L

Tompkins and coworkers (USA) who reported mutants with inactive or temperature sensitive ureases or with very low urease activity.

Molecular biology can also be a valuable epidemiological tool. Examination of the restriction enzyme digest patterns of the ribosomal RNA genes revealed 77 different ribotypes from 100 patients (W Tee, Australia). Sequential isolates from the same patient revealed identical patterns confirming that infection with a particular strain persists. Such a method provides a highly discriminatory typing scheme although it is unsuitable for routine laboratories.

Molecular biology can also aid in detection of very small numbers of organisms as demonstrated by TU Westblom (USA) who detected H. pylori in 1/5 saliva samples from H. pylori-infected patients using the polymerase chain reaction.

What does the urease do?

The urease is likely to be important in colonisation, although urease-negative mutants do colonise, even though infection is delayed. It may also be an important source of nutrients.

Positive correlations between the numbers of H. pylori and the extent of gastritis, and between gastritis and the level of ammonia in the gastric juice, lend support to the idea of ammonia as a pathogenic factor (S Takahashi, Japan).

Studies reported with a urease inhibitor are discouraging for those who see the urease as a therapeutic target. Fluorofamide, the most potent specific urease inhibitor yet described, abolishes the in vitro tolerance of H. pylori to acid in the presence of physiological concentrations of urea (AA McCollm, UK). However in vivo studies in ferrets and monkeys show no effect on the organism despite considerable urease inhibition. The authors suggested that the inhibitor might only be effective during initial colonisation during bacterial transit across the lumen.

One of the most interesting reports concerned the identification of an H. pylori protein homologous with the human heat shock protein, hsp 60 (B Dunn, USA). This

Newell DB
Muirson, P

major surface-exposed protein in H pylori is highly immunogenic (B Dunn, USA). The authors suggested that the H pylori protein contributes to gastric injury by stimulating T cells which cross-react with similar determinants present on endogenous heat shock proteins from stressed host cells.

H pylori produces at least two classes of adhesins: a shared adhesin and hemagglutinins of different specificities (P Doig, Canada). The authors suggested that these adhesins offer goals for therapy by immunisation to provoke an immune response to H pylori adhesins; competitive inhibition with synthetic adhesin peptides; adhesin receptor megatherapy.

An interesting study that may give some clues to the factors influencing colonisation was presented by N Coltro (Australia). Although H. muridarum is normally found in the ileum of infected mice, changes in the gastric environment due to colonisation with H. felis or C. hominis allow it to colonise the gastric mucosa.

Immunisation not promising

Many new serological kits were evaluated, including second-generation kits using combinations of purified antigens. It was stressed that serological assays need to be standardised and validated in each population.

While the immune response may help the clinician in diagnosis or in monitoring therapy, it does not appear to greatly benefit the host. The immune response does not limit infection (although no systemic infection occurs) but may prevent reinfection. However higher IgA levels were observed in patients with less inflammation, suggesting that specific IgA could be in some degree protective against tissue injury in H. pylori infection (GI Perez-Perez, USA).

Immunisation does not appear promising. Parenteral immunisation of specific pathogen free mice with H. felis gave no protection against gastric colonisation; previous oral infection only delayed colonisation (K Heap, Australia). Although intra-Peyers' patch immunisation of killed H. pylori in rats shows that the gut mucosa can mount a vigorous immune response, oral immunisation with either live or killed bacteria induced no significant serum or saliva antibody response (M Dunkley, Australia).

M Blaser (USA) warned that because of the possible autoimmune component of the disease the wrong vaccine could actually make things worse.

Helicobacter mustelae isolated from faeces

Further evidence for transmission within the family came from H Mitchell (Australia). Serial serum samples were taken from children from a low-income area in Mexico City. By the age of 2, 50% of children had H. pylori antibodies with the highest frequency of seroconversion between 5 and 15 months.

The faecal-oral theory received support from data from J Fox's group (USA)

showing isolation of H. mustelae and other Helicobacter species from the faeces of 24/26 9-10 week old ferrets. Negative faecal cultures from the same animals at 20 weeks suggested that organisms were only shed during the period of transient hypochlorhydria following natural infection. However H. mustelae were also isolated from 3/4 8 month old ferrets. These observations suggest a number of possibilities: ferrets with acidic pH can shed the organism; H. mustelae-associated hypochlorhydria can exist for longer periods of time in selected ferrets or H. mustelae is shed in the faeces intermittently.

53% than in rural (38%) populations, suggesting increased probability of person to person spread due to high city population density.

H. felis survived in mucosal scrapings for 5 days at 22 degrees and 2 weeks at 40 degrees without losing infectivity (KS Diker, Turkey).

Australian gastroenterologists are obviously nervous about H. pylori, and rightly so it seems. A second study on H. pylori infection (M Schembri, Australia) in this group showed the prevalence of



Dr. R. Warren (left) receives plaque from Prof. A. Lee in recognition of his role in the discovery of H. pylori.



Earlier suggestions that water supply was a source of infection were not confirmed by H Mitchell (Australia) who found from studies in rural and urban regions in Southern China that water source was not a significant factor in H. pylori infection. Infection was significantly higher in urban

H. pylori infection to be significantly higher than in general physicians, gastroenterology nurses and a control population. Prevalence increased with years of practice with 83% of gastroenterologists who had practised for more than 10 years having H. pylori antibodies!

Antibiotic concentration at mucosa important

The main issues addressed concerning therapy included in vitro sensitivity, the role of acid suppression in eradication and the pharmacology of antibiotics and bismuth compounds, particularly mucosal delivery and new targets.

Y Glupczynski (Belgium) pointed out that in vitro testing should only be used as a first screen - resistance probably predicts poor clinical outcome. In vitro results do not reflect the drug concentration in mucosa nor local conditions at the infection.

A number of those present felt that, contrary to the Working Party's conservative recommendations to only treat duodenal ulcer patients with complications or frequent relapse, all duodenal ulcer patients should be treated. Once the complications have occurred it is too late.

The trend to shorter treatment regimens was illustrated by a report of 47% eradication after 4 days of De-Nol, tetracycline, and nifedipine (Y Glupczynski, Belgium).

The importance of the local concentrations of antibiotics was addressed. Antagonists may decrease mucosal concentrations. Omeprazole, while effective in eradicating H. pylori, may enhance antibiotic efficacy by increasing the local concentration and activity.

W Bar (Germany) showed that bismuth compounds reduced electron flow and that the respiratory chain is the target.

What are the future options for therapy? Current triple therapy may be improved: pH control and manipulating dosages and formulations. There is a need to understand the pharmacology of the gastric mucosa to improve drug penetration. Goals for therapy include altering motility, preventing adhesion and neutralising cytotoxins.

A novel intervention strategy was suggested by work from D O'Connor and A (Australia) who showed in this first demonstration of bacterial interference in the gastric mucosa that C. hominis, isolated from the stomachs of infected humans, does not eradicate H. pylori from humans.

An ad hoc committee was formed in Sydney to develop specific guidelines for in vitro susceptibility testing that can be accepted by all researchers in the field. If you are interested please contact: Dr T. Ulf Wenckebom, Division of Infectious Diseases, St. Louis University School of Medicine, 1402 S. Grand Boulevard, St. Louis, MO 63104, USA or Dr Y. Glupczynski, Brugmann University Hospital, 6 Place van Gehuchten, Brussels 1020 Belgium.

Gastric cancer link with H.pylori becomes stronger

Two papers recently published in the New England Journal of Medicine have dramatically strengthened the hypothesis that past infection with H.pylori considerably increases the risk of gastric carcinoma.

In the last issue of HP Worldwide, the first prospective study (by D Forman and colleagues) linking prior H.pylori infection to subsequent development of gastric cancer was reported. The latest studies in larger groups and in different populations have now confirmed and extended these initial findings.

In the Californian study (Parsonnet et al), 186 patients with gastric carcinoma were selected from nearly 129,000 individuals followed since the 1960's at a health maintenance organisation. The mean interval between serum collection and diagnosis was 14.2 years. Comparison of these patients with matched controls showed that 44% of the 109 patients with confirmed gastric adenocarcinoma had been infected previously compared with 61% of the controls (odds ratio, 3.6). H.pylori infection was a particularly strong risk factor for women (odds ratio, 18, and blacks, 4). A history of peptic ulcer disease was negatively associated with subsequent gastric cancer.

The authors conclude that individuals seropositive for H.pylori were approximately three times more likely to have gastric cancer in the ensuing 1 to 24 years of follow-up than matched controls. In contrast to earlier studies from Parsonnet's group which suggested that intestinal type cancer but not the diffuse type was linked to H.pylori infection, no difference was seen between the odds ratios for the two types of cancer.

Is early infection important?

Only a very small proportion of infected persons ever develop gastric cancer and

Parsonnet et al suggest that to increase the risk of cancer H.pylori infection must begin in childhood. W Haenszel and co-workers had already shown in 1972 that the risk of gastric carcinoma was largely determined by environmental factors in the first few decades of life. H.pylori infection in childhood is well known to be more common in high-risk populations.

H.pylori is a plausible pathophysiological cofactor for cancer, argue Parsonnet et al. Metabolic products of the organisms may directly transform the mucosa, rapid turnover of cells resulting from infection-induced injury may increase the risk of DNA damage, predisposing the mucosa to transformation by ingested or endogenous mutagens; endogenous byproducts of inflammation such as superoxide and hydroxyl ions may cause oxidative damage, mutation, and malignant transformation. Chronic inflammation in other organ systems has been linked with an increased cancer risk, e.g. ulcerative colitis and colorectal carcinoma.

High-risk population

The second paper focuses on a known gastric cancer high risk population. A Nomura and coworkers examined serum samples collected between 1967 and 1970 from 5908 American men of Japanese ancestry in Hawaii. By 1989, 109 cases of pathologically confirmed gastric carcinoma had been diagnosed, 94% of these were positive for H.pylori antibodies compared to 76% of the matched controls (odds ratio, 6.0). As in Forman's study, there was a progressive increase in the risk of gastric carcinoma as the level of H.pylori antibody increased.

Both groups of investigators estimate that approximately 60% of gastric carcinomas in the populations studied could be prevented if H.pylori infection were eliminated.

Association of H.pylori with gastric cancer beyond reasonable doubt

These findings attracted great interest at the recent conferences in Sydney and Amsterdam. "Gastric cancer now belongs in the spectrum of diseases that are associated with H.pylori infection," said Dr David Forman (UK), speaking at the European Digestive Disease Week in Amsterdam.

While atrophic gastritis has long been associated with gastric cancer and epidemiological data have supported the idea of an association with H.pylori, the publication of three follow-up studies in different populations has "changed the situation dramatically," said Forman. "It is now beyond reasonable doubt that there is some association between H.pylori and gastric cancer."

Possible properties of H.pylori that are relevant to carcinogenesis include sustained inflammation, cytokine expression, hypochlorhydria, reactive oxygen intermediates, decrease in gastric juice ascorbic acid and monocyte and macrophage activation. Forman drew attention to the work of Sonata and colleagues (reported in the last issue of HP Worldwide), showing a decrease in the amount of ascorbic acid in gastric juice with H.pylori infection. "H.pylori not only seems to be involved in generating carcinogens in the stomach," said Forman, "it

also suppresses production of the protective natural antioxidants such as ascorbic acid. The entire model of gastric cancer, particularly the link with Helicobacter, is going to have to be rethought."

Dr Forman stressed that other factors are involved in the development of gastric cancer. Strain differences may be responsible for the different outcomes seen between gastric cancer victims that need to be addressed. Further possible strain variation in the influence of the organism on infection links with clinical, epidemiological and histological and primary prevention of infection.

Intervention trial

The next step in the study is evidently obviously an intervention trial, i.e. whether prevention of infection reduces incidence of gastric cancer.

One such trial currently under way being carried out in Venezuela by the International Agency for Research Cancer in association with the World Health Organisation. The Andean region of Latin America is one of the highest risk areas in the world for gastric cancer. Gastric cancer is the leading cause of death from cancer in Venezuela. An extensive screening program has been operating since early 1990.

The aim of the study is to determine whether intervention with the antioxidant treatment of gastric cancer H.pylori can help to reverse the progression of superficial gastritis to more advanced chronic atrophic gastritis and to prevent metaplasia. This series of lesions is generally considered to represent a minimum sequence of normal to cancer.

Most dietary studies have shown a protective effect of a diet rich in fruits and vegetables and this provides the rationale for the use of the antioxidants, vitamin C and E and vitamins B6 and C.

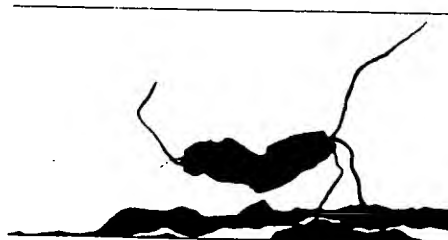
Subjects with a diagnosis of early or above lesions will be randomised to

(continued on page 4)

FIFTH WORKSHOP ON GASTRODUODENAL PATHOLOGY AND HELICOBACTER PYLORI DUBLIN, IRELAND, JULY 5-7, 1992

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MEETING OF THE EUROPEAN HELICOBACTER PYLORI STUDY GROUP DEADLINE FOR ABSTRACTS APRIL 24TH 1992

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receive either anti-H pylori treatment or placebo. At the completion of this phase, the two groups will be further divided into those receiving antioxidant treatment or placebo. Because of the slow progression of precancerous lesions, results will not be available until 1995/6.

Is gastric cancer an infectious disease?

In an editorial accompanying the two latest papers in the New England Journal of Medicine, Dr P Correa (USA) poses the provocative question: Is gastric carcinoma an infectious disease? The cause of gastric cancer is almost certainly multifactorial, says Correa. Three factors appear to be dominant: H pylori infection, excessive salt intake and a diet low in fresh fruits and vegetables. Intervention studies currently under way should help to clarify the role played by H pylori.

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Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. New England Journal of Medicine 1991; 325: 1132-6

Odds ratios for the association of gastric cancer with H pylori seropositivity

Study	Cases/controls	Odds ratio
Forman et al	29/116	2.4
Parsonnet et al	109/109	3.6
Nomura et al	109/109	6.0

Highlights on important developments in research on Helicobacter pylori

1. Graham DY et al.

The addition of triple therapy to standard treatment accelerated healing in patients with duodenal ulcer (15)

2. Bertram EA et al.

Patients from South America had a more severe gastritis than did those from North America. The neutrophil was the predominant inflammatory cell type in South American patients compared to the lymphocyte in North American patients. The authors conclude that the severity and cell

type of H pylori associated gastritis are influenced by geographical factors that may be similar to those that modify infection rates for different geographical locations (113)

3. Karnes WE Jr et al.

Serological data suggest that most patients with atrophic body gastritis, despite having a low incidence of current overt infection, have been infected with H pylori in the past (13)

4. Jones STM et al.

Presence of H pylori antibodies in rheumatoid arthritis patients correlated strongly with a previous history of peptic ulcer disease and with the severity of NSAID-related dyspeptic symptoms. The authors suggest

that H pylori may have a definite role in the pathogenesis of symptomatic peptic ulcer disease associated with more chronic NSAID usage (211)

5. Morris AJ et al.

Arthur Morris describes how the H pylori infection he acquired after deliberately ingesting the organism was finally cleared by the use of triple therapy. Gastritis resolved and serological values returned to normal (14)

6. Blecker U et al.

A case is reported in a 15-year old girl of fatigue, pallor and syncope, but no gastrointestinal symptoms, due to H pylori active haemorrhagic gastritis. On H pylori eradication, symptoms disappeared (38)

7. Oderda G et al.

Recurrent abdominal pain, frequent associated with recurrent vomiting and diarrhoea, is the most common symptom of H pylori infection in children. One third of children have active gastritis, which seems to be an early stage of the disease (2)

8. Sarosiek J et al.

The thickness of the mucous layer in biopsy material from the duodenum, antrum and corpus of H pylori infected subjects significantly thinner than from control subjects (11)

9. Cunn SJ et al.

Oral immunisation of mice and humans with killed H pylori induced specific antibodies

CALENDAR

1992

25 - 27 March

British Society of Gastroenterology Meeting, Sheffield, UK

9 - 14 May

Digestive Disease Week, San Francisco, USA

19 - 23 May

34th Annual Meeting of the Hungarian Society of Gastroenterology, Balaton, Hungary

5 - 7 July

Fifth Workshop on Gastrointestinal Pathology and Helicobacter pylori, Dublin, Ireland

25 - 30 September

1st United European Gastroenterology Week 14th International Congress of Gastroenterology 7th Congress of Digestive Endoscopy, Athens, Greece

23 - 28 October

American College of Gastroenterology, Miami Beach, Florida, USA

12 - 14 November

7th National Symposium of the Italian Society on Digestive Endoscopy, Naples, Italy

27 - 28 November

Meeting on Upper Gastrointestinal Tract Diseases, London, UK

29 November - 3 December

Asian Pacific Association for Gastroenterology, Bangkok, Thailand

1993

14 - 21 May

Digestive Disease Week, Boston, USA

22 - 27 October

American College of Gastroenterology, Washington DC, USA

1994

21 - 27 May

Digestive Disease Week, New Orleans, USA

2 - 7 October

10th World Congresses of Gastroenterology, Los Angeles, USA

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Changing views on duodenal ulcer treatment

"All duodenal ulcer patients should now be offered H.pylori eradication therapy", said Dr Tom Borody (Australia) at a recent symposium in Amsterdam on 'The disease consequences of H.pylori infection in man'. "I feel that in future H₂-blockers will form second line therapy."

While not everyone agreed completely with Dr Borody's recommendations, the majority of the audience attending the symposium came away with changed views on how to treat duodenal ulcer.

"Should we now be concentrating on pH or H₂ on acid suppression or H.pylori eradication", asked Dr Borody. "There is very little doubt that H.pylori eradication virtually abolishes duodenal ulcer recurrence and changes the natural history of the disease", he said, referring to follow-up studies over six years. H.pylori has been shown to be the major causal factor in over 94% of duodenal ulcer patients in his clinic.

"Suppression of acid by H₂-blockers is unphysiological", pointed out Borody. "Relapse rates of 75-89% after stopping therapy may necessitate costly life-long therapy. On the other hand, eradication of H.pylori aims to restore normal physiological conditions. Therapy is cheap, need only last for two weeks and results in accelerated ulcer healing and prevention of ulcer relapse."

Optimising triple therapy

How can triple therapy be optimised? "What is important is the drug concentration at the mucosal surface", explained Borody. "The side effects are probably due to systemic absorption of antibiotics. Individual doses can be lowered by increasing the frequency while at the same time increasing the local concentration by the use of H₂-blockers or proton pump blockers." Dr Borody reported that increasing the frequency of triple therapy dosing to five times a day, halving the tetracycline concentration and reducing metronidazole from 250 to 200 mg, and adding an H₂-blocker at night, resulted in a significant reduction in side effects without any effect on the rate of eradication, around 95%.

What is the future role of H₂-blockers in duodenal ulcer disease? "These drugs will still be used in temporary treatment and in pain reduction during triple therapy", said Dr Borody. "They will continue to be used as maintenance treatment where treatment has failed to eradicate H.pylori and may be used to optimise triple therapy by in-

creasing the local concentration of antibiotics."

How to treat patients in whom eradication has previously failed? Dr Borody uses a custom-built therapy based on drug sensitivity and achieves 70% eradication in this patient group. "Metronidazole resistance is not a significant factor", he claimed. "Eradication rates of 87% are still achieved in the population carrying resistant strains."

"It is going to become unethical not to offer antibacterial therapy to patients with chronic duodenal ulcer", said Dr Borody.

Dr Tony Axon (UK), co-chairman of the symposium, outlined other major unanswered questions. If H.pylori is so widespread why doesn't everyone have duodenal ulcer? How does the organism cause ulcer? What evidence is there that H.pylori may be responsible for gastric cancer?

Professor Martin Blaser (USA) spoke on pathogenic mechanisms involved in H.pylori infection (see page 1) and the role of H.pylori in gastric carcinogenesis was reviewed by Dr David Forman (UK) (see page 4).

Different disease outcomes

Dr Mike Dixon (UK) focused on those morphological features of gastritis that give some insight into the natural history of the disease and disease associations.

Acute neutrophilic gastritis is rarely seen at biopsy. Three months after infection the gastritis has become chronic with infiltrates of chronic inflammatory cells. In only very few individuals does the gastritis resolve. 80% of those with gastritis go on to develop chronic pangastritis, which may develop into atrophic gastritis, both the prevalence and severity of which increase with age. Intestinal metaplasia also increases with age. In wide-spread atrophy and intestinal metaplasia, H.pylori is no longer present.

Chronic pangastritis with continuing epithelial degeneration is associated with gastric ulcer and atrophic gastritis is associated with gastric cancer. "However

the vast majority of subjects have no symptoms", pointed out Dr Dixon.

The remaining 20% of individuals infected with H.pylori develop antral gastritis, which is associated with gastric metaplasia, active chronic duodenitis and duodenal ulcer. The occurrence of gastric metaplasia is not dependent on H.pylori infection but is more extensive in infected individuals. "Gastric metaplasia appears to develop as a response to an increased acid load", explained Dr Dixon.

What determines which of these two patterns of inflammation occurs? Dr Dixon speculated that in individuals with a high intrinsic acid output, the acid in the corpus may protect against colonisation. Such individuals will develop an antral predominant gastritis and may develop gastric metaplasia in the duodenum because of the high acid load reaching the first part of the duodenum. Antral gastritis and gastric metaplasia have long been known as the two major risk factors in duodenal ulcer.

The central feature leading to diminished mucosal resistance is chronic inflammation. The only way to reduce inflammation is to remove its cause, to eradicate H.pylori", concluded Dr Dixon.

The most common chronic infection in man

H.pylori eradication also received attention in other sessions.

There is no doubt that H.pylori has revolutionised our thinking about the etiology and treatment of duodenal ulcer", said JJ Misiewicz (UK), reviewing recent advances in gastroenterology. "The prolonged hypergastrinemia associated with H.pylori infection, the 'most common chronic infection in man', may lead to increased parietal cell mass, hyperacidity and resultant gastric metaplasia in the duodenum and subsequent colonisation of this gastric metaplasia by H.pylori from the stomach", suggested Dr Misiewicz. "Triple therapy is the only way to eradicate H.pylori", he said, "and the trend is towards shorter and shorter treatment regimens." A one week

treatment regimen was reported by RPH Logan (UK). Duodenal ulcers healed in 22/33 patients after only one week of triple therapy and 7 of the remaining 11 went on to heal after this week. The overall eradication rate was 58%.

"Eradication of H.pylori should be considered before surgery", recommended RH Hunt (Canada), speaking on the long-term therapeutic options for peptic ulcer disease.

Is H.pylori involved in the development of refractoriness to H₂-antagonist treatment? C Bianchi Porro (Italy) reported that 96% of duodenal ulcer patients resistant to 4 weeks full dose H₂-blocker treatment: healed after triple therapy compared to 31% after sucralfate. 8/9 patients who did not heal with sucralfate subsequently healed with triple therapy. One month after healing, eradication of H.pylori was 83% in the treated group but nil in the sucralfate group. On the basis of these results Bianchi Porro suggested that first line therapy with triple therapy instead of H₂-blockers should be considered.

Less relapse in gastric ulcer

Most eradication studies have focused on duodenal ulcer. K Seppala (Finland) presented early results showing that H.pylori eradication improves the rate of healing in gastric ulcer. Healing was seen in 92% of cases where H.pylori was eradicated compared to 74% who remained H.pylori-positive. Relapse data confirm what has been shown in duodenal ulcer. None of the 15 patients who remained H.pylori-negative had relapsed after 33 weeks compared to 14/38 who were H.pylori-positive.

Seeing H.pylori in vivo

Direct visualisation of H.pylori on the human gastric mucosa was reported by T Kato and colleagues (Japan), using an in vivo urease test. The patchy distribution of H.pylori on the mucosa has been a problem in diagnosis by biopsy. After premedication with famotidine or omeprazole to adjust the gastric pH, the gastric mucosa was sprayed with a mixture of phenol red and urea by a tube inserted through the endoscope. H.pylori-positive areas changed colour. Biopsies confirmed the presence or absence of H.pylori, with 2/37 false positives and no false negatives.

Seeing H.pylori through the microscope may not be quite so easy. AH Christensen (Denmark) warned of the limitations of histological diagnoses of H.pylori infection by comparing the reproducibility of diagnoses by different pathologists.

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Chapter 17

GONORRHEA VACCINES

John W. Boslego and Carolyn D. Deal

ETIOLOGIC AGENT AND PATHOGENESIS

DESCRIPTION OF THE AGENT

Neisseria gonorrhoeae is the etiologic agent of gonorrhea. The bacterium belongs to the family Neisseriaceae, which includes both pathogenic (*N. meningitidis*) and nonpathogenic (*N. sicca*, *N. subflava*) *Neisseria* (1). The clinical manifestations of gonorrhea, such as genital exudates, have been described for centuries, but it was not until 1879 that Albert Neisser first described the organism in urethral pus (2). *Neisseria gonorrhoeae* was first isolated in vitro in 1882.

Neisseria gonorrhoeae is a gram-negative diplococcus that grows on artificial medium at 37°C in a 5% CO₂ environment. The organism is oxidase-positive and ferments glucose, but not maltose, sucrose, or lactose.

Satisfactory treatment of clinical gonococcal infections was first achieved in the 1930s with sulfonamides (3). However, the organism quickly developed resistance to the drug, which has been a recurring theme. Penicillin was introduced for *N. gonorrhoeae* therapy in the 1940s and was highly successful (4). In the past 40 years, however, the organism has continued to evolve and manifest a variety of drug resistances (5). Penicillin can no longer be used in many regions of the world, including parts of the United States (5). Close surveillance of antimicrobial susceptibility patterns is necessary since the organism continues to develop resistance to the drugs used for its treatment.

CLINICAL MANIFESTATIONS

Neisseria gonorrhoeae usually causes a local mucosal infection, but the spectrum of disease ranges from asymptomatic carriage to disseminated infection. The primary manifes-

tations are urethritis in the male and cervicitis in the female. *Neisseria gonorrhoeae* infects other mucosal surfaces as well, resulting in conjunctivitis (neonatal and adult), pharyngitis, and proctitis.

Local extension of infection occurs in both sexes and is responsible for the major morbidity associated with gonorrhea. In men, the infection may extend to the epididymis, testes, or prostate. These unusual complications can result in sterility.

In the female, local extension of infection is more common, and more serious. Often acting in concert with other organisms, *N. gonorrhoeae* causes endometritis, salpingitis, peritonitis (pelvic inflammatory disease, PID), and perihepatitis. These infections can result in tubal scarring with infertility and subsequent ectopic pregnancy, tuboovarian abscess, and chronic pelvic pain. Estimates are that up to 45% of the women who contract a genital gonococcal infection will develop PID (6). Infertility rates after a single episode of PID approach 15%, and after three episodes, 75% (6).

Disseminated gonococcal infection is manifested by papular or petechial skin lesions (usually on extremities), arthralgias, tenosynovitis, and oligoarthritis. Rarely, myocarditis, hepatitis, endocarditis, and meningitis occur. Strains causing disseminated infection are usually serum-resistant and of a particular auxotype (Arg⁻, Ura⁻, Hyx⁻) (7). Patients who are genetically deficient in one of the terminal complement components are predisposed to repeated episodes of disseminated gonorrhea (8).

EPIDEMIOLOGY/DISEASE BURDEN

Gonorrhea is the most commonly reported infectious disease in the United States (6). Despite the relative ease of diagnosis and treat-

ment, and the extensive public health network for case identification and contact tracing, the epidemic continues.

In the United States, there are approximately 1 million cases reported each year (6). The highest attack rates are in young adults, ages 15 to 24 (6). It is estimated that the reported cases represent only about half of the actual cases. The number of cases peaked in the United States in 1975 and has plateaued and slowly declined since then (7).

Reliable estimates of disease rates worldwide are not available. Because many of the less-developed countries lack the resources for early diagnosis, treatment, and contact tracing, gonorrhea presents enormous health problems in these regions. Epidemic infertility occurs in sub-Saharan Africa and is attributed to gonorrhea (6).

In the United States, the major morbidity and financial costs are related to PID. There are an estimated 400,000 cases of gonococcal PID each year. Considering the total number of cases of gonorrhea, the cost for evaluation and treatment (particularly of PID, infertility, and ectopic pregnancy) approaches \$1 billion/year in the United States alone (6).

PATHOGENESIS

Neisseria gonorrhoeae is a uniquely human pathogen. The organism survives poorly outside the host unless artificially cultured. The disease is spread from person to person, usually by sexual contact with infected secretion. Once the bacteria are deposited on a mucosal surface, a series of events occurs that results in invasion of mucosal columnar cells and a host inflammatory response.

The stages of pathogenesis have been most closely studied in organ cultures of fallopian tubes and entail distant attachment of the organism to the host cell, close attachment and multiplication, ingestion by the epithelial cell, transportation through the cell in phagosomes, possible egestion onto the basement membrane, and, in rare instances, blood-stream invasion (9-11). The epithelial lining is markedly affected by gonococci, demonstrating loss of ciliary motility and extrusion of ciliated cells. The classic exudate consists mainly of host inflammatory cells, denuded epithelial cells, and gonococci.

The contribution of individual components of *N. gonorrhoeae* to each phase of pathogenesis is the subject of considerable study and will be discussed in more detail below. Recent developments in molecular bi-

ology have added substantially to our understanding of these events, but much remains to be learned.

HISTORY OF VACCINATION AGAINST GONORRHEA

Several gonococcal vaccines have been evaluated for efficacy in humans. The first effort was conducted by Greenberg et al. in Canada in the early 1970s (12). Greenberg et al. utilized three seed strains of type I (piliated) gonococci to prepare a killed, autolyzed vaccine. The vaccine was well tolerated and in the majority of volunteers stimulated an antibody response (bentonite flocculation, tissue culture neutralization) that was generally of short-lived. Volunteers received three doses of vaccine, 1 week apart. In the study, conducted in a high-risk population, 62 volunteers were entered into a randomized, placebo-controlled trial. During a 12-month observation period, 10 of 33 vaccine recipients and 7 of 24 control recipients acquired gonorrhea, indicating no protective effect.

Brinton et al. later prepared purified pilus vaccines and tested them in human volunteers in a series of experimental challenge studies (13). The single-pilus vaccine was highly successful in preventing disease when the challenge strain was identical to the vaccine seed strain. The protection could be overcome by higher challenge inocula. Importantly, when a heterologous challenge strain was used, there was no apparent protection (C. Brinton, oral communication, 1982).

A field trial utilizing a purified single-pilus vaccine was conducted in high-risk U.S. military personnel in the Republic of Korea in 1983 (14). In this randomized double-blind, placebo-controlled trial, 3250 volunteers participated. Two doses of vaccine or placebo were administered 2 weeks apart. The observation period was 8 weeks. In male volunteers, 108 vaccine and 101 placebo recipients acquired gonorrhea 2 weeks or more after initial immunization. There was no vaccine protection despite the development of high levels of serum cross-reactive pilus antibody levels.

Lastly, a protein I vaccine was prepared and tested by E. W. Hook III in a human challenge model. The vaccine was well-tolerated and elicited a serum antibody response. This vaccine also afforded no protection against experimental gonorrhea (E. W. Hook III, oral communication, 1986).

VACCINE POTENTIAL OF GONOCOCCAL AN

PILIN OR PEPTIDES

Pili, filamentous projections from one of the major surface antigens of the coccus. Each pilus is composed of a subunit association of the units. This antigenic variation is called a "switch" between the pili. More play tremendous role in the virulence of the amino-terminal region is highly conserved. The amino-terminal region by insertion of four amino acids into the amino acid chain. The amino-terminal pilin is homologous to pilins including (16), *Pseudomonas aeruginosa* (19). All the unusual N-

Several studies have shown that virulence factor is a candidate. It is reported that the virulence model was characterized by morphologies of the pili. The pili are reported to be involved in the attachment of the host cell (21). Electron microscopy has indicated that pili are involved in the attachment of the host cell (21). Electron microscopy has indicated that pili are involved in the attachment of the host cell (21).

Brinton et al. (13) reported that the virulence of the host cell (21). Electron microscopy has indicated that pili are involved in the attachment of the host cell (21).

VACCINE POTENTIAL OF IMPORTANT GONOCOCCAL ANTIGENS

PILIN OR PEPTIDES

Pili, filamentous projections from the cell, are one of the major surface antigens of the gonococcus. Each pilus is formed by the specific association of thousands of pilin protein subunits. This antigen displays both phase and antigenic variation. The organism can "switch" between a piliated and a nonpiliated state. Moreover, the subunits can display tremendous antigenic variation. The amino-terminal portion of the pilin sequence is highly conserved. Antigenic variation occurs predominantly in the carboxy-terminal region by insertions and deletions of two to four amino acid residues as well as single amino acid changes (15). The conserved amino-terminal sequence of the gonococcal pilin is homologous to that of other bacterial pilins including those from *N. meningitidis* (16), *Pseudomonas aeruginosa* (17), *Moraxella nonliquefaciens* (18), and *Bacteroides nodosus* (19). All these sequences begin with the unusual N-methylphenylalanine residue.

Several studies have implicated pili as a virulence factor and as a potential vaccine candidate. Initial studies by Kellogg et al. reported that virulence in a human challenge model was correlated with certain colony morphologies (T1, T2) that were later discovered to represent piliated phenotypes (20, 21). Electron microscopic studies have implicated pili as mediating attachment of the gonococcus to the microvilli of nonciliated columnar epithelial cells (22). The pilus may somehow enable the organism to overcome the normal repulsive electrostatic barrier to attachment between the gonococcus and the host cell (23). Alternatively, a specific receptor-ligand interaction is possible, though no specific eukaryotic receptor for the pilus has yet been identified. Certainly, the presence of pili enhances attachment to a variety of eukaryotic cells. In addition to adherence, piliated gonococci have also been reported to be more resistant to phagocytosis than nonpiliated organisms (24).

Brinton et al. demonstrated that immunization of human volunteers with purified pilin resulted in the generation of antibody that was protective against challenge with the homologous strain (13, 25). In another human infection study, the expression of the pili was again correlated with virulence of the gonococcus, with piliated organisms causing disease and nonpiliated ones being avirulent

(26). More importantly, this study demonstrated the in vivo antigenic variability of pilin. Disease isolates expressed numerous and different pilin types compared with pilin of the input strain. It is this capacity for antigenic variation in vivo that may allow the organism to circumvent a pilus type-specific antibody response.

The molecular mechanisms for antigenic variation have been studied by several laboratories (27-29). The variation is mediated by silent copies of pilin genes of differing antigenic types and locations on the gonococcal chromosome. Gene conversion of these copies into the expression site gives rise to expressed pilin of different antigenic types (30). One method of phase variation is deletion of the pilin gene at the expression site, which results in a nonpiliated phenotype, which can sometimes revert back to a piliated form (29). Recent reports indicate that another mechanism may also be involved, and that is DNA transformation of pilin genes between organisms (31). Thus, the gonococcus displays several sophisticated mechanisms for variation of this major surface antigen.

Selective regions of the pilin sequence, presented to the immune system as synthetic peptides, may make effective immunogens (32). Hopefully, a polypeptide representing only a sequence-conserved region may allow the resultant antibodies to effectively block all gonococci, but that is still speculative at this point. Studies to determine the x-ray crystallographic structure of the pilin molecule should facilitate the identification of exposed regions, which could be effective in this regard (33).

Pili, then, appear to be a major virulence factor for the gonococcus. The concept of a vaccine composed of pilin, or some portion thereof, is of considerable interest, but optimism for its success is dampened by the degree of pilin antigenic diversity and the fact that the human immune response appears directed primarily against the variable portion (14).

NONPILIN ADHESINS

In addition to pilin, Muir et al. have reported the presence of other proteins associated with the pilus fiber (34). These proteins copurify with pilin and may possibly be incorporated into the pilus supramolecular structure. It is suggested that these proteins may be analogous to those seen in uropathogenic *Escherichia coli*, in which proteins incorpo-

rated at the tip of the pilus mediate adhesion to carbohydrate receptors present in the urinary tract (35).

Other studies indicate the possibility of pilin-independent adhesins that may be present on gonococci and mediate binding to eukaryotic cells. Both piliated and nonpiliated gonococci bind to carbohydrate-containing glycolipids (gangliotetraosylceramide and gangliotriaosylceramide) that have been isolated from eukaryotic cells (36). If this function is biologically significant and common among gonococcal strains, then an adhesin protein, or a peptide corresponding to the binding domain, would make a feasible vaccine candidate. However, the role of these proteins in the pathogenic process is unclear at present, and little is known about their potential immunogenicity.

OUTER MEMBRANE PROTEINS

There are three predominant outer membrane proteins (proteins I, II, III) in the gonococcal membrane. The role of these proteins in the pathogenesis of disease is still speculative. Proteins I and II are antigenically variable, while protein III appears to be identical in all strains.

Protein I

Protein I accounts for the majority of protein in the outer membrane and is designated the major outer membrane protein (37). It is found in all gonococci and varies in molecular mass (32–37 kDa) among strains (9). Protein I is believed to function as the porin protein by forming hydrophilic channels through the outer membrane (38). At least a portion of the protein is surface-exposed (39).

The antigenic variability of protein I provides a useful mechanism to classify gonococcal strains. A single strain expresses only one protein I, which remains antigenically stable (9). There are two major subclasses of protein I: protein IA and protein IB. The amino acid sequence of representative strains of each subclass is now known (40, 41). Each subclass represents a family of structurally different, but similar, protein I's. Protein IAs are generally of lower molecular weight. While the amount of protein I that is surface-exposed tends to differ among strains, protein IAs have a smaller surface-exposed portion than protein IBs (42). The surface-exposed determinants form the basis of the current serologic classification schemes. A commonly used system employs six protein IA and six

protein IB monoclonal antibodies (43). On the basis of its reaction pattern to this panel of monoclonal antibodies in a coagglutination assay, a strain can be classified into a serovar. To date, 24 protein IA and 32 protein IB serovars are recognized worldwide. In any given region, however, the overwhelming majority of strains are represented by far fewer serovars.

There are functional correlations between protein I subclass/serovar and characteristics of the organism and/or expression of disease. Protein IAs are associated with disseminated gonococcal infection and resistance to killing by normal human serum (44). Protein IBs are more closely associated with antibiotic resistances (45) and with local mucosal disease (46). However, these associations are far from absolute, and there is considerable overlap. Nevertheless, this typing scheme has been of considerable value in outbreak investigations and in epidemiologic studies of disease transmission (47–49).

Although the role of protein I in the pathogenicity of human infection is not established, there are several lines of evidence that encourage its further investigation as a vaccine candidate.

Protein I is essential for organism survival. It is surface-exposed and invariant in a given strain. There is considerable structural and antigenic similarity among strains in each subclass. It appears to interact at the eukaryotic cell membrane and may trigger endocytosis of the organism by the host mucosal cell (9).

Protein I is immunogenic in humans during the course of a natural infection. Patients develop protein I antibodies in their local secretions and serum as a consequence of infection (50–53). These antibodies exhibit both opsonic and bactericidal properties (54, 55). Moreover, many of the protein I monoclonal antibodies activate complement and lyse the organism (42, 55).

Clinical studies also support the concept that protein I antibodies may protect against infection. Buchanan et al. demonstrated that recurrent episodes of acute gonococcal salpingitis were not caused by gonococci of the same protein I type (56). More recently, Plummer et al. showed that female genital infection with a given serovar appeared to provide protection against a subsequent infection with the same serovar (57).

To date, one protein I vaccine was unsuccessful in a male gonococcal urethritis infection trial, as described previously. While dis-

appointing, this investigation of alternative manufacturing approaches for protein I vaccine is a quicker avenue that lies in the identification of the epitope(s) that is sufficient for functional antibody binding.

The current methods for protein I sequencing and characterization on surface-exposed epitopes for gene cloning greatly enhance our understanding of this important antigen.

Protein II

Protein II is a surface-exposed outer membrane protein that actually describes a family of proteins (protein IIs) that vary among strains. Protein II is at least partially conserved. When expressed, it represents a percentage of the total outer membrane protein.

Protein II expression is in part, with the development of an opaque culture media and a phase-contrast microscope (61).

The antigenic variability of protein II has been the subject of much study. A single strain may express more than one antigenic type of protein II and switch from protein II-negative to protein II-positive (59, 62).

Protein II appears to be involved in adherence proper. Protein II expression is increased in gonococci that have increased adherence to epithelial cells (64). Protein IIs mediate adherence.

The role of protein II in the pathogenesis of disease is unknown. Opaque variation is observed from natural isolates, whereas transparent isolates recovered from infected individuals are not infectious in naive females developing gonorrhea. Protein II is a virulence factor in vivo. In an

appointing, this trial should not impede the investigation of alternative protein I vaccines. Manufacturing a vaccine composed of a mixture of protein I serovars is one approach, but a quicker avenue for vaccine development lies in the identification of a conserved epitope(s) that is surface-exposed and the target of functional antibody activity.

The current or imminent availability of protein I sequence data, structural information on surface-exposed epitopes, and methods for gene cloning and expression will greatly enhance our understanding of this important antigen (40, 41, 58).

Protein II

Protein II is a 24- to 30-kDa heat-modifiable outer membrane protein (9). Protein II actually describes a family of related proteins (protein IIs) that manifest tremendous intra-strain and interstrain variations (59). The protein is at least partially surface-exposed. When expressed, protein II constitutes a high percentage of the outer membrane (60).

Protein II expression is associated, at least in part, with the ability of the organism to develop opaque colony types when grown on culture media and viewed under a dissecting microscope (61).

The antigenic variation of protein II has been the subject of considerable study. A single strain may simultaneously express more than one antigenically different protein IIs and switch from a protein II-positive to protein II-negative phenotype, or from one protein II type to another at a high frequency (59, 62).

Protein II appears to bestow increased adherence properties to gonococci (63). Protein II expression is associated with greater gonococci-gonococci adhesion, as well as increased adhesion of gonococci to human epithelial cells, conjunctival cells, and neutrophils (64). It is possible that different protein IIs mediate adhesion to different cell types.

The role of protein II in human infection is unknown. Clinical studies have shown that opaque variants are more commonly recovered from mucosal gonococcal infections, whereas transparent variants are more often recovered from asymptomatic or disseminated infections (65). Protein IIs are immunogenic in natural infection. Both males and females develop antibodies, but their antibacterial properties are not known (50, 66). Antigenic variation of protein IIs also occurs in vivo. In an experimental infection study, a

broad array of protein II variants appeared during the course of infection after a predominantly protein II-negative phenotype was instilled intraurethrally (67).

Protein II is currently not considered an attractive vaccine candidate. Although it is surface-exposed and quantitatively significant in protein II-positive organisms, the bacteria can survive and grow in its absence. Even though a pathogenic relationship is suggested by its adherence properties, too little is currently known to establish a defined role in human infection. Lastly, the phenomenal array of antigenically distinct protein IIs would make it a very difficult antigen to incorporate into a vaccine.

Protein III

Protein III is a 30- to 31-kDa outer membrane protein (9). It is surface-exposed and present in all strains of gonococci (68). Moreover, an analogous protein, designated class 4, also exists in *N. meningitidis* (69).

Protein III is closely associated with protein I in the bacterial membrane, but its function in bacterial physiology or in the pathogenesis of disease is not known (69). There is no evidence to support its role as a porin protein or as a cofactor for protein I in this capacity.

In stark contrast to other gonococcal surface antigens, there is no evidence for structural or antigenic variation in protein III (70). All available studies suggest it is invariant among gonococcal strains. Moreover, protein III shares remarkable sequence and structural similarity to the Omp A proteins of *Enterobacteriaceae*, particularly *E. coli* (71). This homology is especially marked in the carboxy portion of the molecule (69).

Despite its surface location, protein III is poorly immunogenic in humans during natural infection (50, 66). Patients demonstrate either no response or low levels of antibody.

The most fascinating aspect of protein III is its apparent capacity to induce and/or bind to antibodies that block the bactericidal activity of antibodies to other surface antigens (protein I, lipopolysaccharide) (72, 73). There is experimental evidence that protein III antibodies (IgG) fix complement, but the resultant membrane attack complex is either defective or incapable of fully inserting into the cell membrane to cause bacteriolysis (74, 75).

Due to its antigenic similarity to Omp A proteins, patients may develop protein III antibodies as a result of colonization/infection

by *Enterobacteriaceae*. These cross-reactive protein III antibodies may then shield the gonococcus from the bactericidal activity of other antibodies. Protein III might then exist on the gonococcus as a mechanism for its own protection. If this scenario proves valid, protein III might be deleterious if incorporated into a vaccine preparation.

Considerable work is in progress to resolve these important issues. The recent construction of a protein III-deficient gonococcal strain will significantly aid in this evaluation (69).

LIPOLYSACCHARIDE

Lipopolysaccharide (LPS) is a major constituent of gram-negative outer membranes and is known to serve several important biological and pathogenic functions. In addition, various LPS epitopes, distinguishable by monoclonal antibodies, are expressed on different gonococcal strains or on the same strain at different times, resulting in LPS antigenic variation.

Phenol-extracted LPS has been shown to mediate most of the toxic damage that occurs during infection of human fallopian tubes (76). The LPS is a target for bactericidal antibodies and regulates complement activation on the bacterial cell surface (77, 78). The presence or absence of certain LPS epitopes may be involved in the determination of serum-sensitive or serum-resistant phenotypes (79).

The structure of gonococcal LPS is similar to that of enteric bacteria in that both have a lipid A fatty acid chain embedded into the cell wall, and a core oligosaccharide linked to three 3-deoxymanno-2-ketooctulosonic acid (KDO) moieties. The gonococcus differs from enteric bacteria, however, in that it lacks an O side chain of strain-specific polysaccharide residues (80). Silver staining of periodate-oxidized LPS and rapid isolation methods have enabled the determination of LPS molecular masses ranging from 3.2 to 7 kDa among strains (81). The development of LPS monoclonal antibodies has allowed the immunological characterization of specific LPS components (79). Recently the structural determination for the oligosaccharide portion of the gonococcal LPS has been proposed (82). Studies by Mandell et al. have shown that particular gonococcal LPSs have carbohydrate structures that are analogous to human erythrocyte glycolipids, and that these two structures cross-react immunologically (83).

The antigenic variation of gonococcal LPS

structures was seen on passage in vitro (81). This variability was also demonstrated in vivo during a human challenge study in which the strains isolated from the infected patient expressed different LPS antigenic types from the challenge strain (84).

An LPS-based vaccine would necessitate the detoxification of the endotoxic-producing properties of LPS. In addition, given the antigenic diversity of the LPS, a constant oligosaccharide portion or a type correlated with virulence would have to be identified. The immunogenicity of this molecule in humans and its apparent role in pathogenesis designate it as another attractive vaccine candidate (77).

H.8 EPITOPE

An epitope contained on two different neisserial lipoproteins that binds to a specific monoclonal antibody is called H.8. The epitope itself appears to be conserved and stable (85). Following its identification in 1984, H.8 gained wide attention because of its presence on pathogenic *Neisseria* (*N. gonorrhoeae*, *N. meningitidis*), but absence on commensal *Neisseria* (85).

The two lipoproteins that contain the H.8 epitope are the lipid-modified azurin (Laz) and the H.8 outer membrane protein (Lip) (86).

The lipid-modified azurin is present in both pathogenic and commensal *Neisseria* (86). It is not reactive with the monoclonal antibody on Western blots. Like other azurin proteins, this lipid-modified azurin may function in electron transport during bacterial respiration. Its role, if any, in pathogenesis is not known.

The H.8 outer membrane protein is also a lipoprotein, but it is present on pathogenic *Neisseria* only. Its apparent molecular mass varies from 22 to 30 kDa among strains (87). This protein is alanine- and proline-rich and does not stain with Coomassie blue after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The H.8 outer membrane lipoprotein has been extracted and purified from neisserial organisms (87, 88), and the gene has been cloned and sequenced (89). The protein consists of a repeating heptapeptide. Differences in the number of repeating units may account for the difference in apparent molecular weight among strains. The function of Lip in the outer membrane is unknown, but it is believed to serve in a structural role.

The H.8 epitope appears to be surface-ex-

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posed on viable gonococci, but electron microscopy studies with gold-labeled monoclonal antibodies have provided conflicting results (85, 86, 90).

Patients develop serum antibodies to the H.8 epitope after local genital infections, salpingitis, or disseminated gonorrhea (50, 51, 91). One patient was found to have H.8 antibody in seminal plasma (50). However, H.8 antibodies have also been found in the sera of patients without a prior history of gonorrhea. One study showed that the acquisition of *N. meningitidis* throat carriage can result in the development of H.8 antibody in the serum (50). In several studies, patients developed local gonococcal infections despite the presence of H.8 antibody in the serum (50, 66).

Most monoclonal antibodies specific for H.8 lack bactericidal activity and fail to protect against meningococcal infection in animal models (92). However, one H.8 monoclonal antibody has been shown to be bactericidal and opsonic for some gonococci (86). Affinity-purified human antibodies specific for H.8 were found to lack bactericidal activity for meningococci (93).

The value of the H.8 epitope as a gonococcal vaccine candidate is unclear. The apparent surface-exposure, stability, and association with pathogenic *Neisseria* make it worthy of pursuit; however, the lack of a known role in pathogenesis and the inability of serum antibody to prevent infection weigh against it.

IGA PROTEASE

Immunoglobulin A represents a major host defense system against microbial pathogens that come in contact with mucosal surfaces (94). However, many bacteria, such as *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, and *Streptococcus pneumoniae*, are known to produce a protease that cleaves human IgA1 at the hinge region (94). These IgA1 proteases are thought to act as a mechanism for bacteria to evade specific mucosal immunity. The construction of IgA1 protease-negative mutants of *N. gonorrhoeae* provides a valuable tool to investigate the significance of this enzyme in the pathogenesis of gonorrhea (95).

The gene encoding the gonococcal IgA1 protease has been cloned. Significant homology with IgA1 protease genes of other species was also found (96). Patients with local gonococcal infections, salpingitis, and disseminated gonorrhea infrequently produce antibody to IgA1 protease in their sera (97). Patients with meningococcal disease or me-

ningococcal carriage more commonly produce specific antibody to the enzyme (97). This antibody is cross-reactive with IgA1 protease from *N. gonorrhoeae* and inhibits the protease activity of the enzyme (97).

The IgA protease presents an attractive vaccine candidate if its biological role in disease is validated. As part of a multicomponent vaccine, antibody raised to this enzyme may well allow IgAs of other specificities to be more efficacious in attacking the organism.

MAJOR IRON-REGULATED PROTEIN

Neisseria gonorrhoeae expresses several proteins under iron-limited conditions (98). These proteins may be involved in iron uptake by the bacteria and therefore may have a function in pathogenesis. In contrast to many gram-negative bacteria that produce soluble siderophores, the gonococcus must obtain iron directly from specific iron-binding proteins (lactoferrin and transferrin) of the host (99).

The major iron-regulated protein (MIRP) is a 37-kDa protein and appears to be common among all gonococci and meningococci (100). Morse et al. have recently described its purification and characterization (101). Antibodies to MIRP have been detected in patients with disease, indicating that it is expressed in vivo (102). This protein is reported to bind iron from transferrin (101).

The apparent immunogenicity and conserved nature of the MIRP makes it an attractive vaccine candidate. Other proteins that mediate iron acquisition also deserve attention. Interference with iron utilization could potentially alter the course of disease.

OTHER GONOCOCCAL ANTIGENS

Anaerobic or aerobic growth conditions induce strains to selectively express a variety of membrane proteins. Other conditions of environmental stress result in the expression of another class of proteins called *stress proteins*. The role of these proteins in organism survival or pathogenesis is not known. Yet it is interesting to note the presence of antibodies to an anaerobically induced protein in women with PID, thus suggesting its immunogenicity and expression in vivo (103).

Gonococcal outer membrane protein-macromolecular complex is a surface-exposed homopolymer. It is antigenically conserved and constitutes about 10% of the outer membrane protein (104). Antibodies raised to this antigen in animals exhibit bactericidal activ-

ologous protection to urethral challenge after parenteral immunization with purified pili.

The ideal vaccine would be one that prevents colonization and local infection. Given the uniquely human reservoir of *N. gonorrhoeae*, such a vaccine may eventually lead to eradication of the organism. If prevention of local infection is not possible, an alternative approach would be a vaccine that limits extension of infection and prevents PID. Such a vaccine would have a profound impact on the morbidity of gonorrhea.

The evaluation of vaccine candidates must proceed cautiously. Should a vaccine allow or predispose to asymptomatic carriage, it might have a deleterious public health effect. Increasing the reservoir of carriers would predictably result in a higher incidence of gonococcal disease, including PID, among the unimmunized.

A successful vaccine will likely be one that induces antibodies that simultaneously attack the organism at several stages of pathogenesis. One might envision the success of vaccine-induced antibodies that allow human IgA survival, promote opsonization and bacteriolysis, and yet block organism adherence, iron usage, and eukaryotic membrane integration.

Appropriate models to evaluate pathogenesis and immunity are a problematic area. Numerous animal models have been employed, but only the chimpanzee urethritis model bears any resemblance to human disease. The availability, difficulty, and cost of these animals have curtailed the use of this model. Other animal models such as the guinea pig chamber and chick embryo are also in use, but their relevance to human disease is unknown.

Tissue and organ cultures are in selective use. Much about pathogenic mechanisms has been learned from the fallopian tube system.

Ultimately, the human will serve for the final validation of a successful vaccine. Human challenge studies have been of considerable value in the past for investigations on pathogenesis and for vaccine testing. The model is safe, and the experimental infection closely resembles naturally acquired disease. More studies are needed in this model to understand the contribution of specific gonococcal components to human disease and to investigate the human immune response. Ethical considerations limit this model to male urethritis only, so the evaluation of vac-

cines that might prevent pelvic inflammatory disease will have to take place in the field.

Ongoing and needed investigations that have the greatest bearing on vaccine development lie in the following three areas:

1. Studies on the molecular basis of pathogenesis that increase our understanding of the critical steps leading from organism exposure to infection are essential. Particular emphasis should be placed on the contribution of individual antigens to this process. Identification of gonococci-host cell interactions, as well as host cell receptors, are a critical phase of these studies.
2. Studies on the extent of antigenic variation, the mechanisms for its control, and the contribution of the host environment and immune response will allow a realistic assessment of potential vaccine candidates. The discovery of common, stable, and functional epitopes in this sea of variability holds the brightest hope for an effective vaccine.
3. Studies on the local immune response in the human genital tract, methods to enhance it, and the value of specific antibodies in protection are vital to our continued progress. Investigations should continue on local immunization procedures.

Many new technologies are now widely available to aid in these investigations. Gene cloning, DNA and protein sequencing, protein structure studies, synthetic peptides, new adjuvants, epitope mapping, the polymerase chain reaction, and the construction of defined mutants can now be brought to bear. It will likely require the close collaboration of many groups with diverse skills in order for us to be successful in the quest for an effective gonorrhea vaccine.

Note: The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

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The development of new techniques in molecular genetics has expanded the number of approaches that can be used for making vaccines. In some cases, established vaccines can be improved or their supply increased. In other cases, new vaccines can be developed that have not been feasible through the application of old technologies. In this regard, it is worth considering first the broad categories into which vaccines can be divided. "Live" vaccines are defined by the ability of the vaccine strain, i.e., of the virus, to replicate within the human host. Conversely, "killed" vaccines ("non-live" may be more accurate, even though most scientists use the term "killed") are unable to replicate or infect the host. Table 29-1 summarizes the salient features of these two categories of vaccines.

Live Vaccines

Live vaccines are attenuated with respect to their ability to cause disease, meaning that they are less likely to cause clinical illness than the natural disease-causing agent. By virtue of their ability to undergo limited replication in the host, such vaccines, typically viruses, often induce cell-mediated (T cell) immunity in addition to antibody-mediated (B cell) immunity. As a result of such a broad spectrum of immunity as well as re-exposures to the virus which silently boost immunity, protection following a single inoculation

with a live attenuated vaccine often lasts a lifetime. However, the ability of the live vaccine to replicate can be detrimental; being genetically plastic, a replicating virus can revert to a more pathogenic form and cause adverse reactions in a vaccinee or a contact of a vaccinee. Sufficient data must be obtained in animal studies as well as in clinical studies to rule out the possibility of reversion.

A number of strategies have been employed for developing live viral vaccines that are attenuated, as summarized in Table 29-2.

Several of these approaches were possible before the development of modern techniques in recombinant DNA (rDNA) technology which enable the manipulation of viruses on the molecular level. These classic approaches, which utilize routine techniques in cell culture, include attenuation in cell culture, selection for temperature-sensitive or cold-adapted viruses, isolation of closely related viruses from other species and selection for reassorted viruses from the progeny of an infection by two parental viruses. (These strategies are discussed in greater detail elsewhere with respect to particular vaccines.)

The ability to alter directly the structure of viruses on the molecular level is enabling scientists to design attenuated vaccines rather than forcing them to rely upon phenotypic selection and upon chance to provide the only mechanisms for viral change. Through techniques of viral genetics and DNA sequence analysis, it is possible to identify those regions in the viral genome where alteration can contribute to the attenuation of viral pathogenicity. This rDNA technol-

Table 29-1. General Characteristic of Vaccines

"Live" Vaccines	
Attenuated with respect to pathogenicity	
Cell-mediated immunity in addition to humoral immunity	
Longer-lasting protection	
Tendency to reactogenicity	
Ability to revert	
"Killed" Vaccines	
Nonreplicating	
Noninfectious	
Lower reactogenicity	
Need for boosters	
High purity	

Table 29-2. Strategies for the Development of Attenuated Live Viral Vaccines

"Classic" Approaches	
Modified by passage in cell culture	
Variant viruses from other species	
Temperature-selected mutants	
Reassorted genomes	
"Molecular" Approaches	
DNA modification mutants	
Recombinant viruses	

ogy allows such regions to be altered or deleted and introduced into the genome of a wild-type virus, thus leading to the production of an attenuated virus. This approach is presented in the schematic in Figure 29-1.

The salient feature of this approach is the deliberate construction of an attenuated virus that is unlikely to revert to a more pathogenic form. This construction is made possible by deleting a portion of a key region of the genome in such a way that reversion is ruled out. This approach first was applied successfully by Kit and coworkers to the attenuation of pseudorabies virus, thus leading to the creation of a safer vaccine for the prevention of a severe disease in pigs.¹ This is the first genetically altered live vaccine that was licensed for use in any species. A related approach is being taken for poliovirus and is applicable to other vaccines for humans.²

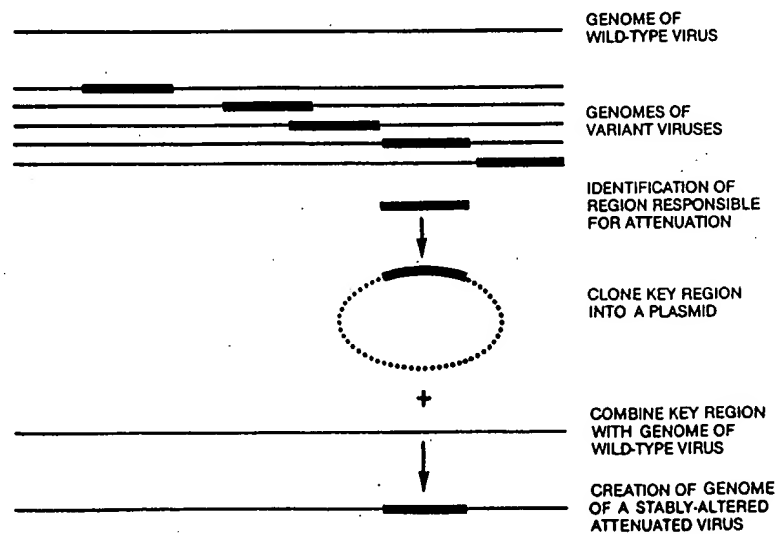
A second approach is the genetic alteration of a live virus to function as a vector, *i.e.*, carrier, for other genes. This approach enables the recombinant virus to function as a vaccine for two or more infectious agents in a single inoculation. This technology first was applied to vaccinia virus.^{3, 4} Prior to this application, wild-type vaccinia virus had been used for the worldwide eradication of smallpox and is the prime example of a variant virus from another species used as a vaccine for humans. A region of the genome of vaccinia virus was identified as nonessential for viral replication by the general approach outlined in Figure 29-1. Within a plasmid containing this nonessential region, a gene encoding a surface protein of another pathogen was inserted (Fig. 29-2). This recombinant plasmid was introduced

together with wild-type virus into cells in culture, resulting in the creation of a recombinant virus that carries the foreign gene.

For insertion into a virus vector, a gene is selected that encodes an immunogen, usually a surface protein, of a virus or a microbial parasite. In order for this strategy to be effective, the presentation of this immunogen during the course of viral replication should result in a protective immune response directed to the antigen and, therefore, the pathogen. Recombinant vaccinia viruses have been derived that express immunogens for hepatitis B virus, herpes simplex virus, influenza virus, rabies virus, Epstein-Barr virus and respiratory syncytial virus. Some of these recombinant viruses have shown promise in animal studies. A similar approach has been taken with respect to the genetic engineering of two human herpesviruses as viral vectors, herpes simplex virus⁵ and varicella-zoster virus.⁶

Table 29-3 outlines several points that are important to the safety and efficacy of such live recombinant vaccines. A nonessential (*i.e.*, not required for viral replication) region for the insertion of a foreign gene often can be used that will result in the attenuation of viral pathogenicity.⁷ Multiple foreign genes can be inserted into a single viral genome, resulting in an immune response against multiple pathogens.⁸ The level of expression of the foreign protein should be high enough to elicit effective immunity. The parental (vector) virus should be tested extensively; its use as a vaccine should be free of side effects. In that regard, the use of the smallpox vaccine strain of vaccinia virus has raised concern with respect to the neurological and dermatological sequelae observed in small numbers of vac-

Figure 29-1. Attenuation of viruses using modern techniques in molecular biology.



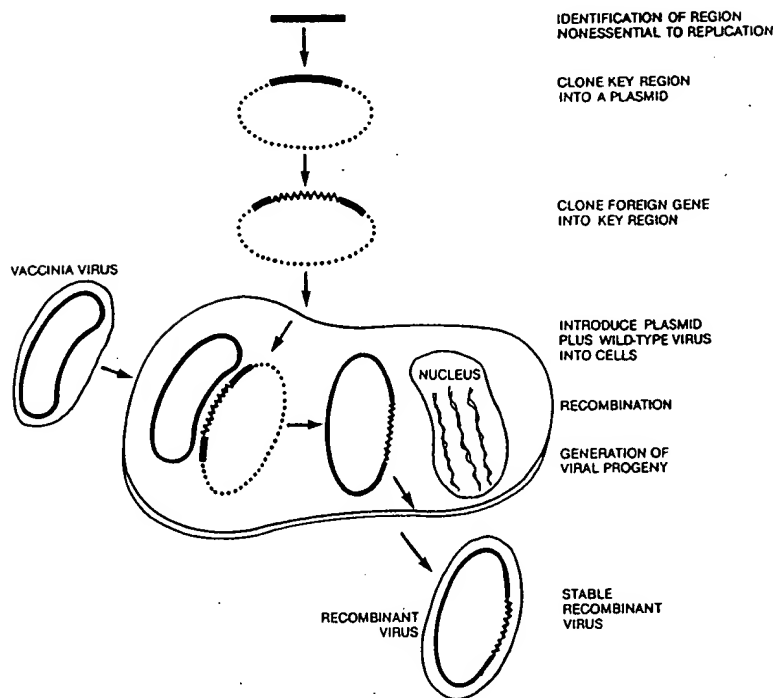


Figure 29-2. Creation of recombinant vaccinia viruses carrying genes that encode immunogens of other pathogens.

cinees. The host range or tissue tropism of the recombinant virus should not be altered significantly compared with that of the vector virus. The effects of viral infection upon the replication and structure of host cells should be studied closely. Since vaccinia virus encodes a protein with significant homology to transforming growth factor- α and to epidermal growth factor (EGF) and since the virus infects cells through the EGF receptor,⁹ which is itself highly homologous to the *erb-B* oncogene, there is concern that infection with vaccinia virus may be mitogenic (stimulates growth or division of infected cells). Finally, while some recombinant vaccinia viruses have shown promise in preclinical testing in models of efficacy in animals, only clinical trials and testing of protective efficacy in humans, still awaited, will permit a complete assessment of the utility of such vaccines.

Table 29-3. Considerations in the Safety and Efficacy of Recombinant Live Vaccines

Safety	
Extensive testing of parental virus	
Stable attenuation of parental virus	
Insertion point for the foreign gene	
Host range of the recombinant virus	
Biology of the cellular receptor for the virus	
Efficacy	
Multiple foreign genes in a single virus vaccine	
Level of expression of foreign protein	
Clinical testing	

Killed Vaccines

In contrast to live vaccines, killed vaccines do not replicate in the host. Consequently, killed vaccines are often less efficient in the induction of cell-mediated immunity. In order to achieve complete and long-term protection, booster inoculations are required. Furthermore, the greater antigenic mass required for a killed vaccine to be effective, when compared with the antigenic mass for a live vaccine, raises issues of purity. Since they do not replicate, killed vaccines cannot revert to cause clinical disease. Several strategies have been used to develop killed vaccines, as summarized in Table 29-4.

The classic approaches, which employ techniques of biochemical purification and biophysical inactivation, include physical inactivation of whole viruses or bacteria, utilization of inactivated toxoids from bacteria, purification of mon-

Table 29-4. Strategies for the Development of Killed Vaccines

"Classic" Approaches	
Killed whole pathogens	
Toxoids from pathogens	
Purified surface components	
Conjugated surface components	
"Molecular" Approaches	
Recombinant-derived proteins	
Synthetic peptides	
Anti-idiotypic antibodies	

omeric or aggregated surface components of viruses or bacteria and conjugation of surface components of bacteria to other molecules. (These strategies are discussed in greater detail elsewhere.)

The techniques of rDNA have revolutionized biomedical research. They make it possible to identify the gene encoding any protein of interest and to insert that gene into a host cell in such a way that the cell can produce large amounts of the particular protein (Fig. 29-3).

This technology is directly applicable to the development of vaccines. The key to the problem is the identification of that protein component of a virus or microbial pathogen that itself can elicit the production of protective antibodies, such antibodies having the capacity to neutralize infectivity and thus protect the host against attack by the pathogen. The protein then defines biochemical tools for research (e.g., antibodies and amino acid sequences), which are useful for the identification and cloning of the gene encoding that protein. Ultimately, the gene is placed into a host cell in a configuration that will result in synthesis by the host cell of large amounts of the particular immunogenic protein.

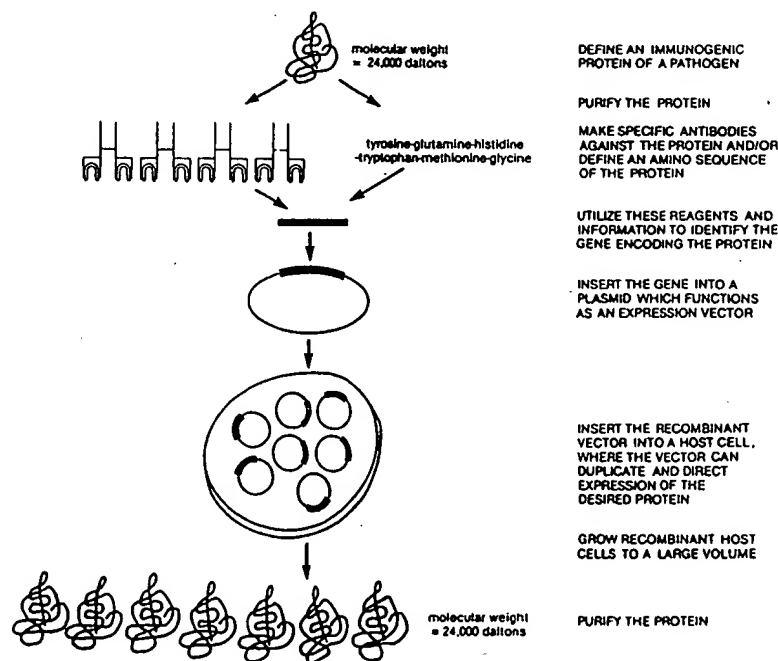
The initial application of rDNA technology to the development of vaccines for humans was for the vaccine to prevent infection by hepatitis B virus (HBV). A safe and effective vaccine, consisting of particles of the surface antigen of HBV (HBsAg) has been prepared from human plasma. In order to expand the available supply of vac-

cine, scientists turned to rDNA technology for vaccine production. The process was initiated by the identification of the gene encoding HBsAg and the insertion of that gene into various host cells. Recombinant yeast synthesize large amounts of particles of HBsAg that are morphologically (Fig. 29-4) and immunologically highly similar to the plasma-derived HBsAg.¹⁰

Recently, the yeast-derived HB vaccine produced by Merck, Sharp & Dohme became the first rDNA-derived vaccine of any type for humans ever to be licensed anywhere in the world. This prototype vaccine offers hope for the development of a new generation of vaccines, including ones for diseases such as malaria^{11, 12} and leprosy¹³ for which vaccines cannot be made using classic technologies. The development of recombinant vaccines ultimately may be facilitated by the application of new techniques for the enhancement of the immunogenicity of isolated proteins; one such technique is hydrophobic aggregation.¹⁴ However, because of the biology of the disease or the nature of the immune response induced by the vaccine, it is important to realize that recombinant vaccines do not always provide the solution to the problem of prevention of an infectious disease.

There are a large number of host cells that can be utilized for the production of rDNA-derived proteins. The most common host cells have been bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*) and mammalian cells (Chinese hamster ovary, monkey kidney). Recently, scientists

Figure 29-3. The use of recombinant DNA (rDNA) technology to express large amounts of a desired protein.



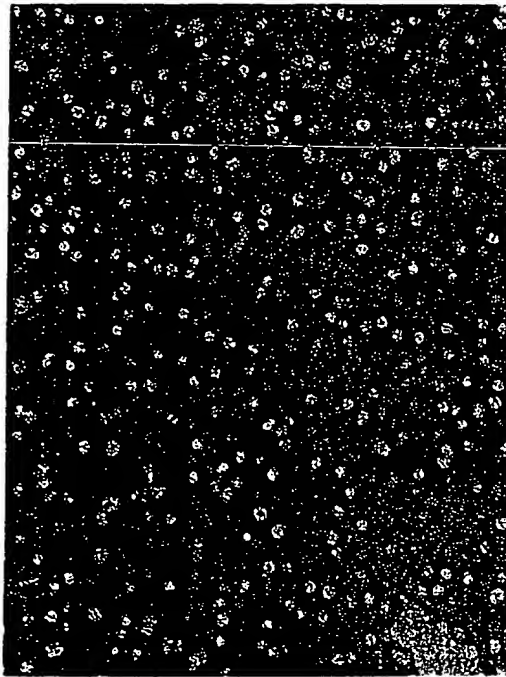


Figure 29-4. Electron micrograph of particles of HBsAg produced by recombinant yeast (165,000X). (Courtesy of B. Wolanski, Merck Sharp & Dohme Research Laboratories.)

have diversified to the use of other bacterial (*Bacillus subtilis*), fungal (*Aspergillus nidulans*) and higher eukaryotic (insert) cells. All these systems can be judged by a wide range of criteria relating to desirable traits of either the protein product or the host cell as well as to safety considerations (Table 29-5).

The most commonly employed expression systems can be evaluated relative to one another

Table 29-5. Expression Systems for rDNA-derived Proteins

Desirable Traits of the Product
High yields (commercial)
Stability of yield with scale-up of cells
Inducible expression
Secretion
Post-translational modifications (consistent with immunogenicity)
Glycosylation
Phosphorylation
Amidation
Carboxylation
Hydroxylation
Proteolytic processing
Desirable Traits of the Host Cells
Ease of scale-up
Consistency of performance
Lack of oncogenic elements
Rapid division
Safety Concerns
Heterologous protein contaminants
Biology of cell substrate
Residual DNA (oncogenesis)

*Scheme to take cultures from bench to large-scale fermentation or purification.

with respect to each of these criteria (Table 29-6). These criteria fall into three groups, which roughly discriminate between the microbial (yeast and bacteria) and mammalian expression systems as follows:

1. The microbial systems are more productive and consistent in overall performance than the mammalian ones.
2. Mammalian cells provide for post-translational modifications that often resemble more closely those in the viral agent than those provided by microbial cells.
3. With few exceptions, serially propagated

Table 29-6. Comparison of Commonly Used Expression Systems for rDNA

	<i>E. coli</i> (Bacteria)	<i>S. cerevisiae</i> (Yeast)	Chinese Hamster Ovary (Mammalian Cells)
Yield of product	+++	+++	+
Ease of scale-up	+++	+++	+
Stability of yield with scale-up	+++	+++	+
Inducible expression	+++	+++	+
Consistency of performance	+++	+++	+
Secretion	+	++	+++
Glycosylation	-	++	+++
Proteolytic processing	-	++	+++
Other modifications	-	++	+++
Biology of cell substrate	++	+++	+
Heterologous protein contaminants	++	++	+
Residual DNA	+++	+++	+

+++ = most acceptable

++ = acceptable

+ = least acceptable

- = absent

mammalian cells, unlike microbial cells, are considered "transformed," meaning that they are more susceptible to oncogenicity in experimental animals.

Furthermore, for expression of rDNA, mammalian cells often utilize genetic elements derived from oncogenic or latent viruses, while microbial cells do not utilize such elements. These perceived safety concerns must be addressed regarding the use of mammalian cells as an expression system for recombinant vaccines.

These relative evaluations represent generalizations from a large number of studies in the different systems and should be considered whenever an expression system is utilized. Nevertheless, each attempt at expression must be evaluated individually, and there are probably as many exceptions as there are rules in the "expression game"!

The use of synthetic peptides as vaccines involves the use of short segments of a protein molecule, rather than the entire molecule, as the immunogen. Some peptides are able to induce antibodies that can react with the whole protein as well as with the peptide per se.¹⁵ The discovery process for formulating synthetic peptide antigens begins by defining the gene encoding the immunogenic protein (see Fig. 29-3), then branches off by exploiting the DNA sequence of the gene to define the amino acid sequence of the protein and to predict which regions of the protein might be immunogenic (Fig. 29-5). Once defined, peptides can be synthesized chemically¹⁶ and formulated into synthetic vaccines.

This approach first was applied to the development of vaccines for humans by synthesizing portions of the HBsAg polypeptide.¹⁷ In theory, the approach is technically versatile and lends itself to the production of well-defined vaccines. However, in practice, the approach has several shortcomings relative to the use of whole proteins. In general, the antibodies elicited by an intact protein crossreact more effectively with both the protein and the pathogen on which it resides than do antibodies elicited by a synthetic peptide. Furthermore, such antibodies bind with higher affinity and are present at a higher titer than are those elicited by the peptide. Thus, the duration of the immune response stimulated by a synthetic peptide is inferior to that stimulated by a whole protein. At minimum, a complete cocktail of synthetic peptides may be required as well as an improvement in methods for the enhancement of immunogenicity by covalent conjugation onto carrier proteins. It may be that synthetic peptides, however tailored, cannot mimic all the conformations assumed by the

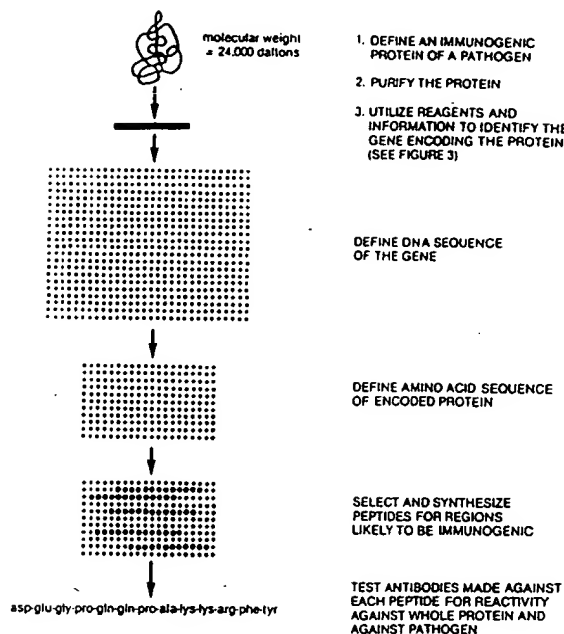


Figure 29-5. Defining immunogenic peptides from immunogenic proteins.

intact protein that are critical for immunogenicity. Furthermore, immunogens often have complex chemical structures, e.g., sugars and lipids, which cannot be specifically applied to a synthetic peptide. However, synthetic peptides may be useful for the priming of an immune response, as first demonstrated for poliovirus.¹⁸

A third novel strategy for the formulation of killed vaccines is the use of anti-idiotypic antibodies (anti-antibodies), whose existence and function in the regulation of the immune response first were articulated by Jerne.¹⁹ Since antibodies bear a structural image of the primary antigen at the antigen-combining site (idiotype), antibodies to antibodies (anti-idiotypic) have an antigen-combining site that is structurally similar to the antigen (Fig. 29-6). Thus, inoculation of the anti-idiotypic antibody functions as a vaccine by inducing an anti-anti-idiotypic antibody which in principle should be identical to the first antibody.²⁰

This approach has been applied to formulating a vaccine for hepatitis B.²¹ While this strategy clearly warrants further study, it suffers from two potential drawbacks. Since the immunogen is an antibody, which is structurally related to naturally occurring human antibodies, problems related to antigenic sensitization must be addressed. In addition, the images borne by anti-idiotypic antibodies are structurally analogous to peptide domains on the surface of the pathogen rather than to whole proteins. Therefore, such

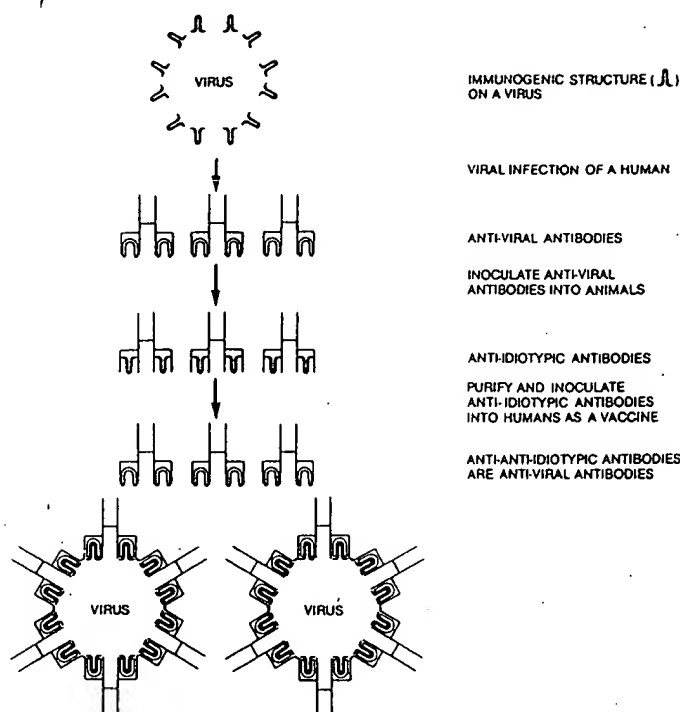


Figure 29-6. Strategy for the use of anti-idiotypic antibodies as vaccines.

vaccines might elicit an immune response which is more antipeptide-like in nature rather than antiprotein-like, as discussed previously.

The progression of a vaccine candidate from the laboratory to the marketplace is long and arduous, often taking 10 years from the time of its initial discovery and characterization. Vaccines made by means of new molecular technol-

ogies are being developed rapidly. Of these, only rDNA-derived proteins have gone as far as human clinical trials, much less having become a licensed product as in the case of yeast-derived HBsAg. Representatives of such vaccines are listed in Table 29-7, along with others derived by older types of technologies.

With the development of the increasingly so-

Table 29-7. The Progression of Human Vaccines Made by Different Technologies Toward Becoming Licensed Products

	Preclinical Testing	Clinical Testing	Licensed Product	Examples
Live Vaccines				
Classic Strategies				
Modification in cell culture	X	X	X	Measles, mumps, rubella
Variants from other species	X	X	X	Smallpox (vaccinia), rotavirus
Temperature-selected mutants	X	X		Influenza
Reassorted genomes	X	X		Rotavirus
Molecular Strategies				
DNA modification mutants	X			Poliovirus, <i>Salmonella</i> , <i>Shigella</i>
Recombinant viruses	X			Vaccinia, herpes simplex, varicella-zoster
Killed Vaccines				
Classic Strategies				
Killed whole pathogens	X	X	X	Pertussis
Toxoids from pathogens	X	X	X	Diphtheria, tetanus, cholera
Purified surface components	X	X	X	Hepatitis B
Conjugated surface components	X	X		Meningitis (<i>Hemophilus influenzae</i> b)
Molecular Strategies				
Recombinant-derived proteins	X	X	X	Hepatitis B
Synthetic peptides	X			Hepatitis B
Anti-idiotypic antibodies	X			Hepatitis B, rabies

phisticated analytical tools of molecular biology and immunology, vaccines derived from the newer technologies are receiving closer scrutiny at the regulatory and clinical levels than have vaccines derived from more classic strategies. This trend is expected to continue and represents a formidable barrier for manufacturers to hurdle with respect to the licensing of safe and effective vaccines. As with any technology, there is a learning curve for both manufacturers and regulatory agencies.

A major challenge facing manufacturers and society in the United States is the profound increase in litigation over adverse experiences related to vaccines. The most dramatic manifestation of this litigation is the increased expense and intermittent unavailability of product liability insurance to the three United States-based manufacturers of the vaccine for pertussis, thus resulting in the temporary withdrawal of products of two of these firms from the market and the tripling in the price of the vaccine. Medically, one may find a situation in which the general welfare of the pediatric population may be at significant risk to whooping cough. This problem could have tragic consequences for society and become a severe disincentive for the development of vaccines by means of new technologies. It is hoped that, while legislative remedies to this severe problem are being addressed, research scientists and medical researchers will continue to receive as much support as possible in the pursuit of new technologies, since the vaccines that result represent the most cost-effective products for the eradication of infectious diseases.

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PLOTKIN & MORTIMER

VACCINES

Ellis

VACCINES

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When Does Homology Mean Something Else?

Molecular biologists routinely compare sequences between different genes, searching for degrees of "homology" between the two; "foul" cry evolutionary biologists, who say the word is misused, thereby causing confusion

"WHEN I use a word," said Humpty Dumpty in a rather scornful tone, "it means just what I choose it to mean—neither more nor less." But, in the scientific realm at least, such an Alice Through the Looking Glass approach to language can lead to misunderstandings: a prime example is the word homology.

"Homology" has the precise meaning in biology of 'having a common evolutionary origin,' but it also carries the loose meaning of 'possessing similarity or being matched.' Its rampant use in the loose sense is clogging the literature on protein and nucleic acid sequence comparisons with muddy writing and, in some cases, muddy thinking."

This verbal volley, launched in the pages of *Cell*, is the most recent attack on an old problem. "Yes, this battle has been fought for more than a decade, but it has usually been fought by individuals," says Gerald Reeck of the Kansas State University. "I thought it was time for a more concerted effort." That effort is in the form of an appeal signed by Reeck and ten prominent evolutionarily-oriented molecular biologists, including, Richard Dickerson, Thomas Dukes, Emanuel Margoliash, and Emile Zuckerkandl. "With a collective effort to mend our ways, proper usage [of homology] would soon become fashionable and therefore easy," they write. "We believe that we and our scientific heirs would benefit significantly."

The problem arises in the comparison of sequences, either of proteins or genes, in which, say, a 20% identity of sequence is typically described as 20% homology. "Molecular biologists know what they mean by such a statement," says Walter Fitch, a molecular biologist at the University of Southern California and a cosigner of the letter. "But in fact they are mixing together two different, but related, properties. To classical biologists, homology means not just similarity of structure, it means common descent. It may be true in many cases that similarity of sequences between, say, two genes is the result of common descent, of homology. But I believe it is important to distinguish

the observation from the conclusion."

Russell Doolittle, a molecular biologist at the University of California at San Diego, and a cosigner of the letter, traces the abuse of homology back through almost two decades. "Most people in protein chemistry in the late 1960s were not classically trained biologists," he explains, "and to them the word homology simply meant similarity."

"When I use a word," said Humpty Dumpty in a rather scornful tone, "it means just what I choose it to mean—neither more nor less."

This word mutation became fixed in this group, and continues through to today. A second group led by Walter Fitch and others—people with a much greater awareness of evolutionary biology—saw the need for correct usage, and invented all kinds of other terms that simply made the whole thing very complicated and esoteric."

In recent years the sequencing fraternity has far outweighed Fitch and his ilk, and homology meaning similarity has become common usage, as any quick glimpse of the literature reveals. "We've been swamped," says Reeck. At the same time, many molecular biologists have become interested in evolutionary questions. As the Whitehead Institute's David Baltimore once remarked, "everything we look at is evolution." Once this happened, the potential for confusion became acute.

The first skirmish over the use of homology was between Fitch and others in the pages of *Science* some 15 years ago. Fitful exchanges continued in various vehicles, with a flurry of letters breaking out in *Nature* 4 years ago. Reeck's current multi-authored appeal is an attempt to raise the issue beyond the level of individual sparring.

"I've been spending summers collaborating with Chrisoph de Haen and David Teller," says Reeck, "and we would discuss this from time to time. Eventually we decided that a group effort was the only way to achieve anything. The result was the letter to *Cell*. De Haen and Teller, both at the University of Washington, became cosigners."

"People tell us that things have gone too far," says Fitch, "even people who were sympathetic with what we are trying to do. Maybe that's true. I'm not interested in fighting for lost causes. I just think things should be clear."

The clarity Fitch seeks is this. "In its precise biological meaning, 'homology' is a concept of quality. The word asserts a type of relationship between two or more things," write Reeck and his colleagues. That relationship is common descent, and therefore homology cannot be partial—10%, 20%, and so on. "Things can't be partially homologous any more than a woman can be partially pregnant," quips Fitch.

"If using 'homology' loosely did not interfere with our thinking about evolutionary relationships," write Reeck and his colleagues, "the way in which we use the term would be a rather unimportant semantic issue. The fact is, however, that loose usage in sequence comparison papers often makes it difficult to know the author's intent and can lead to confusion for the reader (and even for the author)."

One key source of confusion is that a degree of structural similarity is an irrefutable, quantified fact, supposing the sequencing has been done correctly. By contrast, the suggestion of common descent must always be an hypothesis, however strongly supported by the evidence. Structural similarity and homology are clearly very closely tied together, but they are not necessarily the same thing.

Keeping the two things apart requires using different words, urge Reeck and his colleagues. "Sequence similarity" should be used to describe what is observed between two structures. "Homology" is the inference of common evolutionary origin. Period. The cosigners say that many people argue that attempting to enforce such terminology is anachronistic, that the word homology itself is evolving and taking on new meanings. "If that evolution is toward vagueness and if it results in making our scientific discourse unclear, surely we should intervene." ■

ROGER LEWIN

ADDITIONAL READING

G. R. Reeck *et al.*, "Homology in proteins and nucleic acids: A terminology muddle and a way out of it," *Cell* 50, 667 (1987).

Letter to the Editor

"Homology" in Proteins and Nucleic Acids: A Terminology Muddle and a Way out of It

"Homology" has the precise meaning in biology of "having a common evolutionary origin," but it also carries the loose meaning of "possessing similarity or being matched." Its rampant use in the loose sense is clogging the literature on protein and nucleic acid sequence comparisons with muddy writing and, in some cases, muddy thinking.

In its precise biological meaning, "homology" is a concept of quality. The word asserts a type of relationship between two or more things. Thus, amino acid or nucleotide sequences are either homologous or they are not. They cannot exhibit a particular "level of homology" or "percent homology." Instead, two sequences possess a certain level of *similarity*. Similarity is thus a quantitative property. Homologous proteins or nucleic acid segments can range from highly similar to not recognizably similar (where similarity has disappeared through divergent evolution).

If using "homology" loosely did not interfere with our thinking about evolutionary relationships, the way in which we use the term would be a rather unimportant semantic issue. The fact is, however, that loose usage in sequence comparison papers often makes it difficult to know the author's intent and can lead to confusion for the reader (and even for the author).

There are three common situations in which hazards arise by using "homology" to mean similarity. The first case is the most obvious offense but perhaps the least troublesome. Here an author identifies sequence similarities (calling them homologies) but claims that the sequences being compared are not evolutionarily related. Some awkward moments occur in such a paper, since the author claims both homology (i.e., similarity) and nonhomology (i.e., lack of a common ancestor). Nonetheless, the author's ideas are likely to be clear since arguments against common ancestry are presented explicitly.

A second case is one in which an author points out similarities (again called homologies) but does not address the issue of evolutionary origins. The reader, seeing the term "homology," may infer that the author is postulating coancestry when that is not the author's intent.

The final case occurs most frequently and is the most subtle and therefore most troublesome. Here, similarities (called homologies) are used to support a hypothesis of evolutionary homology. In this case, the two meanings of homology seem to overlap, and it is almost inevitable that the thinking of author and reader alike will be intrusively distorted as follows. Similarity is relatively straightforward to document. In comparing sequences, a similarity can take the form of a numerical score (% amino acid or nucleotide positional identity, in the simplest approach) or of a probability associated with such a score. In comparisons of three-dimensional structures, a typical numerical

description is root-mean-square positional deviation between compared atomic positions. A similarity, then, can become a fully documented, simple fact. On the other hand, a common evolutionary origin must usually remain a hypothesis, supported by a set of arguments that might include sequence or three-dimensional similarity. Not all similarity connotes homology but that can be easily overlooked if similarities are called homologies. Thus, in this third case, we can deceive ourselves into thinking we have proved something substantial (evolutionary homology) when, in actuality, we have merely established a simple fact (a similarity, mislabeled as homology). Homology among similar structures is a hypothesis that may be correct or mistaken, but a similarity itself is a fact, however it is interpreted.

We believe that the concepts of evolutionary homology and sequence or three-dimensional similarity can be kept distinct only if they are referred to with different words. We therefore offer the following recommendations:

- Sequence similarities (or other types of similarity) should simply be called similarities. They should be documented by appropriate statistical analysis. In writing about sequence similarities the following sorts of terms might be used: a level or degree of similarity; an alignment with optimized similarity; the % positional identity in an alignment; the probability associated with an alignment.

- Homology should mean "possessing a common evolutionary origin" and in the vast majority of reports should have no other meaning. Evidence for evolutionary homology should be explicitly laid out, making it clear that the proposed relationship is based on the level of observed similarity, the statistical significance of the similarity, and possibly other lines of reasoning.

One could argue that the meaning of the term "homology" is itself evolving. But if that evolution is toward vagueness and if it results in making our scientific discourse unclear, surely we should intervene. With a collective decision to mend our ways, proper usage would soon become fashionable and therefore easy. We believe that we and our scientific heirs would benefit significantly.

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Advances in vector systems for gene therapy

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Innovation in gene therapy has become an area of intense interest to biomedical researchers, due to the failure of conventional methods to provide safe, prolonged expression of genes in vivo. Transfection methods provide safe, efficient delivery, but only transient expression. Although viral transduction methods provide permanent insertion of genes into the genome (via retroviruses), they are subject to safety problems, complement inactivation, size limitations and transcriptional inactivation of the viral promoter. This void between the two methods is being fulfilled by new materials and procedures aimed at utilising the best of both transduction and transfection manoeuvres. Synthetic vectors and delivery systems are also being devised to permit more selective targeting on three levels: transductional targeting (entry into desired cells); transcriptional targeting (selective expression); and positional targeting (entry into precise genomic loci). The tools for these advanced molecular conjugate vectors include combinations of polycations, peptide signals, liposomes, viral enzymes, and the appropriate nucleic acid substrates.

DRUG DESCRIPTORS:

*dna

liposome--pharmaceutics--pr; polycation--pharmaceutics--pr; virus enzyme

MEDICAL DESCRIPTORS:

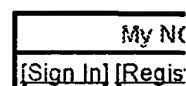
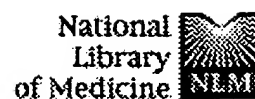
*gene therapy; *cloning vector

genetic engineering; genetic transcription; genetic transduction; medical research; methodology; nonhuman; patent; review; genetic transfection; virus; pharmaceutics; drug delivery system

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Gene therapy for cancer.

Culver KW, Blaese RM.

Human Gene Therapy Research Institute, Iowa Methodist Medical Center, Des Moines 50309.

Initiation of clinical trials of gene therapies for cancer has been made possible by two major technological advances: the ability to clone genes that constitute the genetic basis of carcinogenesis or that have therapeutic potential, and the development of an increasing number of gene transfer methods. As a result, 30 experimental trials of gene therapy for the treatment of human cancer have been approved in the United States of America. Here, we discuss the current status of gene therapy for cancer together with future directions for its development.

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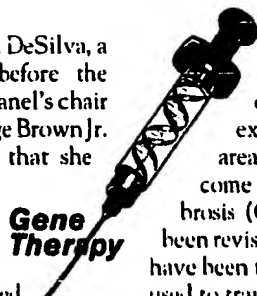
Gene Therapy's Growing Pains

With more than 100 clinical trials started and hundreds of millions of dollars at stake, the field is struggling to meet expectations

Last September, when Ashanthi DeSilva, a cheerful 8-year-old, appeared before the House Science Committee, the panel's chair at the time, Representative George Brown Jr. (D-CA), was moved to declare that she was "living proof that a miracle has occurred." DeSilva made history in 1990 when she received the first authorized human gene therapy ever attempted. She had been born with a defective version of the gene that normally makes the essential enzyme adenosine deaminase (ADA)—a condition that, left untreated, causes a fatal malfunction of the immune system. Four years after receiving her first injection of cells containing functioning ADA genes, Ashanthi, apparently in good health, was chatting with members of Congress.

Since that epic treatment, gene therapy has taken off like a rocket. More than 100 clinical trials, aimed at treating conditions ranging from inherited disorders such as cystic fibrosis to cancer and AIDS, have been given the go-ahead. The National Institutes of Health (NIH) is spending an estimated \$200 million a year to develop and test tools and techniques for gene therapy. Private companies have raised hundreds of millions of dollars to enter the field and are now sponsoring most of the clinical trials. Many academic centers have created gene-therapy programs and joined the jockeying for a piece of the action.

Yet in spite of this enthusiasm—bolstered by media hype—all is not well in the world of gene therapy. So far, there has been no unambiguous evidence that genetic treatment has produced therapeutic benefits. Even data from the pioneering ADA trials are not decisive: Ashanthi and the other children who have since been treated with gene therapy are also being given routine injections of synthetic ADA, and these conventional treatments may be responsible for their good health (see box on p. 1051). Gene therapists are still encour-



tering difficulties in transferring genes to adequate numbers of target cells and getting them expressed. This problem afflicts all areas of gene therapy, but it has become acute in efforts to treat cystic fibrosis (CF): Several CF protocols have been revised because of side effects that may have been triggered by the adenovirus agent used to transfer genes, and some researchers say that adenovirus-based therapy for CF must now be rethought (see box on p. 1052).

Faced with such fundamental problems, several biomedical leaders, including NIH Director Harold Varmus, are saying it's time for NIH to pause, examine what gene therapy has accomplished, and determine what role NIH should be playing in the field. "Despite the growing support for gene therapy," Varmus said at a public meeting in May, the field "remains at a very early stage of development. While there are several reports of convincing gene transfer and expression, there is still little or no evidence of therapeutic benefit in patients—or even in animal models." Nor, he added, is there a consensus about which gene delivery systems will be most effective, and he said he wasn't confident the field was choosing the best lines of attack.

Of particular concern to Varmus and some leaders in the field is the possibility that the intense commercial interest in gene therapy is prompting a stampede into clinical trials and pressure for quick results—before the basic science has been worked out. Drew Pardoll, a Johns Hopkins University co-investigator in a gene-therapy trial for prostate cancer, deplores the lack of rigor in many studies. "There's been an emphasis on glitz," he says. "It's produced a culture in which getting into clinical trials—getting into the club—has been more important than getting a meaningful result."

These concerns have prompted the most intensive review of this burgeoning field since that first ADA experi-

ment 5 years ago. Earlier this year, Varmus created two high-level panels to advise him on how NIH should proceed. The first, chaired by Inder Verma, a geneticist at the Salk Institute, is looking into NIH's procedures for approving gene-therapy clinical trials (see box on p. 1054). The second, co-chaired by Arne Motulsky, a geneticist at the University of Washington, Seattle, and Stuart Orkin, a hematologist at Harvard University, has been asked to chart a strategy for how NIH should invest in gene therapy, choose areas to emphasize, and help shape guidelines for medical practice. Both panels will issue recommendations by December.

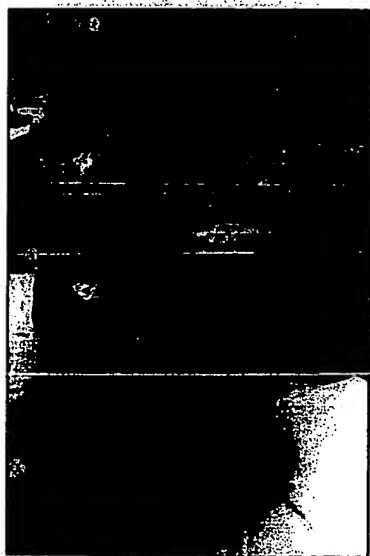
The Motulsky-Orkin panel is drawing a lot of interest—and some nervousness—from gene-therapy researchers in part because Varmus deliberately set it up to take an independent look at the field. Varmus chose its members, he said, for their "stature in the scientific community" and because none is directly involved in running a gene-therapy company or clinical trial.

Varmus's intramural adviser on gene therapy, virologist Nelson Wivel, director of the NIH Office of Recombinant DNA Activities, says he "would not be surprised" if the panel suggests backing off from the heavy emphasis on clinical trials today. Instead, Wivel suggests, the panel may stress the importance of funding basic virology and immunology. "This is the primary question," Wivel says: "Should you be doing more [clinical] trials before you've solved other major technical issues," such as making vectors more efficient and less toxic? These recent developments at NIH, the cradle of gene therapy, suggest the soaring enthusiasm for clinical experimentation may be cooling.

A glass half full?

That enthusiasm is still very visible these days—particularly in the media. "Gene Therapy Techniques Advance as Potential Treatments for Cancer," reported *Genetic Engineering News* on 1 March. "The Birth of a Megamarket," proclaimed *Fortune* on 15 May, featuring Canji Inc., a gene-therapy company in San Diego. "Gene Therapy May One Day Help Doctors Fix Ailing Hearts," announced Johns Hopkins University on 28 July. "Gene Therapy Boosts Radiation Therapy for Cancer," said a University of Chicago press release on 31 July.

Beginning with a wave of media attention



High hopes. The first attempt, in 1990, to correct an ADA gene defect.

JOHN CRANFORD/NIH

Jury Still Out on Pioneering Treatment

Every time physician Melissa Elder opens a vial of the enzyme she injects into two young brothers she treats, it costs \$2200. Elder says the two boys use a total of five vials a week; it costs more than \$40,000 a month to keep them healthy.

These brothers—Rhett, age 4, and Zach, age 2—lack a gene that expresses the enzyme adenosine deaminase (ADA), essential to the immune system. Failure to produce ADA leads to a deadly condition: severe combined immunodeficiency disease. To fend it off and keep infection at bay, Elder, an immunologist at the University of California, San Francisco, treats Rhett and Zach with a synthetic form of the enzyme known as PEG-ADA.* She says the parents are acutely aware of their sons' vulnerability—and of the cost of using PEG-ADA: "The parents lose sleep worrying about what will happen when their insurance reaches its cap." The policy has a limit of \$1 million, already half spent.

This is exactly the kind of misfortune gene therapy is meant to prevent. But it hasn't in this case: Zach has received gene therapy to replace missing ADA genes since shortly after he was born. Like the other children who have been given ADA gene therapy in the United States and overseas, he still gets weekly injections of PEG-ADA. Even the two girls who made history 5 years ago as the first patients to receive ADA gene therapy receive PEG-ADA shots. The reason: Physicians have seen other children's immune function decline when PEG-ADA was reduced, and they worry that it would risk the children's health to rely on gene therapy alone.

Elder and other physicians treating the handful of children who have been given gene therapy for ADA deficiency say their patients' health has improved. But as long as the children continue to get PEG-ADA shots, researchers cannot say for sure how much of the credit should go to the gene therapy.

Even principal investigators in the gene therapy trials—Michael Blaese of the National Institutes of Health (NIH) and Donald Kohn of the Los Angeles Children's Hospital—agree that the mixed treatment clouds the role of gene therapy. "There are a lot of questions to be answered," Blaese concedes. But he argues that, in the case of his first two gene-therapy patients, "the experiment was valuable irrespective of whether [the children] were on enzyme or not." He says the experiment proved that it's possible to transfer corrective genes to humans and to get the genes to express ADA "at a very good level" in at least one patient—Ashanthi DeSilva—for several years.

Ashanthi was given her first dose of gene therapy in 1990; a second patient was treated in 1991. Both were also put on PEG-ADA, approved as a standard therapy in 1990 by the Food and Drug Administration. In attempting gene therapy, Blaese and a team at NIH focused at first on T cells circulating in the girls' bloodstream—removing blood, treating T cells with stimulants, inserting a new ADA gene, and infusing the cells back into the patients. Each girl received 11 to 12 treatments. Blood tests conducted 3 years later showed that more than 50% of Ashanthi's circulating T cells contained the new gene, says Blaese.

But, in a telling indication of the hit-or-miss nature of this new technology, only 0.1% to 1% of the other patient's did. Clinical signs have improved in both girls, however. In Ashanthi's case, "it's very hard to say this was due just to enzyme [PEG-ADA]," says Blaese, although he recognizes that in the other case, "there just isn't enough" of the new gene present for it to deserve much credit.

Other researchers say it's easy to overestimate gene therapy's contribution. Ricardo Sorensen, a physician at the Louisiana State University Medical Center who treats ADA-deficient children, notes that the infusion of stimulated T cells alone may have been beneficial for these young patients and that the PEG-ADA must have helped. The best way to sort out what each treatment did, says Sorensen, would be to give T cell therapy, PEG-ADA therapy, and gene therapy independently to patients with similar conditions. Short of that, says Michael Hershfield, the Duke University researcher who developed PEG-ADA, a good way to get an answer would be to withdraw PEG-ADA from children who have received gene therapy and see how they do. That is

exactly what Blaese and his colleague Kohn are doing right now.

Together, they have been running an experiment in which Zach and two other ADA-deficient boys were given a new type of ADA gene therapy in 1993, at their birth. In all three cases, researchers removed blood from the children's umbilical cord and attempted to inject an ADA gene into long-lived "stem" cells, which give birth to other blood cells and are relatively abundant in cord blood. The goal: to create a permanent source of ADA-competent T cells. Preliminary data suggest partial success: Up to 10% of their circulating T cells now seem to carry a healthy gene, and Kohn says the hope is that, with time, these healthy cells will accumulate and predominate.

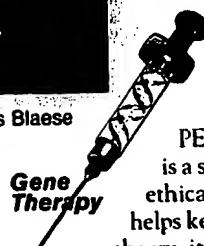
These children have also been receiving PEG-ADA since birth, says Kohn, because "it is a standard therapy, and we felt it wouldn't be ethical to withhold it." However, the PEG-ADA helps keep genetically defective cells alive, and in theory, its use retards the rate at which they can be cleared from the bloodstream to make room for healthy cells. For this reason, Blaese and Kohn are eager to see the boys' PEG-ADA shots curtailed. Since January, Blaese says, the level of PEG-ADA given these three patients has been cut in half (to 30 units per kilogram per week). By the end of the year, he had hoped to cut it close to zero.

But the experiment is not advancing as rapidly as Blaese would like. Physicians for all three boys—including Elder—say they are reluctant to cut the PEG-ADA doses below the present level. Elder, for example, says: "The more PEG-ADA I give, the better the white cell count" and the stronger the immune function. Already the patients' white cell counts have dropped with the initial decline in PEG-ADA doses, although the fraction of "cured" T cells has increased. Physicians are watching closely to see whether the boys can tolerate further reductions before allowing the experiment to proceed. If so, and if the transplanted genes eventually provide all the ADA Zach and the other two boys in this test require, it would be the first unambiguous demonstration that gene therapy has cured a patient's disease.

—E.M.



Proof seeker. NIH's Blaese aims to prove ADA therapy works.



* Polyethylene glycol-ADA, bovine ADA with artificial surfaces added to prolong life in the bloodstream, manufactured as Adagen by Enzon Inc. of Piscataway, N.J.

generated by NIH's attempt to fix Ashanthi DeSilva's defective ADA gene 5 years ago, encouraging reports like these have swelled to a flood. Most such reports are based on research developments that have yet to be tested in clinical trials, however. And the clinical trials that have been conducted over the past 5 years have yielded very few published results—so few that the Motulsky-Orkin panel will have little hard data to analyze as it tries to figure out how the field is progressing.

NIH's Recombinant DNA Advisory Committee (RAC), which reviews all NIH-funded clinical research protocols for gene therapy, discovered for itself the paucity of data when it established a subcommittee to see where the field is heading. The panel, led by Brian Smith, a Yale University oncologist and RAC member, and NIH staffer Debra Wilson, scanned all trials approved by the

RAC and the Food and Drug Administration (FDA) through June 1995. The panel found little concrete information on the results of these trials, but it did paint a remarkable picture of how rapidly the field has grown—both in terms of the numbers of trials and the wide range of disorders gene therapists are boldly trying to treat.

The RAC team found that 567 patients are involved in 106 RAC-approved experiments. Almost half (268) are new recruits, having entered trials since December 1994. Only a small fraction of these experiments are aimed at correcting defective genes. Instead, most protocols aim to induce specific cells, such as cancer cells or cells infected by HIV, to produce proteins that would make them vulnerable to attack by the immune system. Others are attempting to use gene therapy as an adjunct to traditional chemo-

therapy for cancer (see chart on next page).

The field, in short, has moved a long way from the popular notion of gene therapy as a cure for genetic disease. Indeed, the RAC panel identified only 20 trials focusing on single-gene deficiencies such as ADA. Of these, 11 aim to replace the defective chloride transport gene that causes cystic fibrosis, using an adenovirus vector to shuttle functioning genes into a patient's lung cells. Three other trials aim to treat Gaucher disease, a metabolic disorder; single trials are aimed at each of six other rare diseases including ADA deficiency. Little has been published from these efforts: Only preliminary data have seen the light of day in peer-reviewed journals.

In contrast to the few efforts aimed at single-gene disorders, almost half the 106 trials are aimed at cancer. One reason for the

The Trouble With Vectors

Cystic fibrosis (CF) is a lethal inherited disease for which gene therapy offers a rare hope of relief. CF patients—of whom there are more than 30,000 in the United States—lack a gene that enables cells to process the chloride ion, causing their lungs to be plagued by mucus and infection. Gene therapy's promise is that one day it may be possible to replace defective genes with healthy ones, lengthening the lives of CF patients, who generally die as children or young adults.

Already, researchers have transferred a working gene (known as CFTR) into the surface airway cells of lab animals. This success has inspired 11 human trials. But any expectation that these tests would quickly demonstrate therapeutic benefits has dwindled as researchers have run into problems in transferring sufficient quantities of the CFTR gene into patients' cells. In addition, the virus vector they are using as the transfer agent has provoked an immune reaction in some patients.

CF researchers are not alone in encountering such difficulties. Indeed, right from the start, gene therapists have recognized that their central challenge would be to find safe vectors capable of transporting genes efficiently into target cells—and getting the cells to express the genes once they are inserted. Although there have been promising developments in some areas, it remains the central challenge for every area of gene therapy. Francis Collins, director of the National Center for Human Genome Research (NCHGR), sums up the situation bluntly: "None of the currently available techniques is clinically useful for systemic gene delivery," the kind that can provide a permanent cure. For that reason, NCHGR has joined in a major program to improve vectors and make them available to clinicians (*Science*, 11 August, p. 751).

The most popular vector used so far is one based on a retrovirus that normally infects mice. A crippled version of this retrovirus, loaded with therapeutic genes, has been used in 76 of the 106 human trials approved to date, most of which involve patients with cancer or HIV. This mouse virus is the most efficient agent yet identified for transferring genes, although rates of transfer and expression vary dramatically in different patients (see p. 1051).

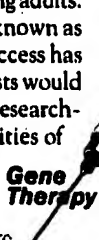
The stark variation among patients isn't the only problem. Another is that retroviruses insert genes only into cells that are actively dividing and growing, such as T cells. This rules out their use for treating diseases such as CF, where the target cells aren't dividing. A second drawback is that retroviruses insert themselves randomly

into host DNA, posing what's thought to be a small—but real—risk of cancer. If a retrovirus gene should settle alongside an oncogene or tumor suppressor gene, it might trigger tumor formation by turning on or off the native gene. For these reasons, retroviral vectors have been used in "ex vivo" procedures—in which cells are removed from the patient, treated, and replaced—and when increased risk of cancer is not considered an obstacle to therapy.

In contrast to those trials, therapy for CF patients has relied primarily on a vector based on a crippled adenovirus. This DNA virus infects 75% of young people, usually without causing illness, according to adenovirus expert Harold Ginsberg, emeritus of Columbia University, now at the National Institutes of Health (NIH). It's attractive for CF therapy because it seeks the lungs. It penetrates nondividing cells and relies on these host cells to express viral DNA.

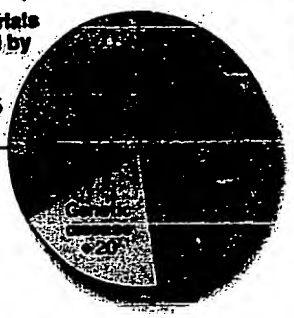
But it, too, has drawbacks. Adenovirus genes express proteins that trigger immune responses. In consequence, large concentrations of wild virus—and even crippled virus—provoke inflammation along with an immune attack that neutralizes cells containing adenovirus genes. For this reason, the effects of adenovirus vector therapy are likely to be short-lived, lasting about 6 weeks. And, because the immune system "remembers" antigens and attacks them with extra vigor on a second encounter, repeat dosing with adenovirus vector seems impractical at present—unless a strong immune response is desired, as in some types of cancer therapy.

Research on CF gene therapy by Richard Boucher and colleagues at the University of North Carolina, Chapel Hill, indicates that clinicians using adenovirus as a vector are caught between two problems. When administered at low concentrations it is inefficient: The virus doesn't get into many human nasal cavity or airway cells, and few cells express the corrected CFTR gene. At high doses, however, it appears to cause acute inflammation, Boucher says. He notes that three or four CF gene therapy trials have been compelled to stop or adjust doses to deal with acute reactions in patients. Some researchers, such as Ronald Crystal, who pioneered this field at NIH and is now at the Cornell University Medical Center in New York, think past problems with CF therapy may not involve fundamental issues so much as a need to find the right way to deliver existing materials. But Boucher and Ginsberg believe immunogenicity has been and continues to be a fundamental problem.



Clinical Trials
Approved by
the RAC
through
June 1995

Arthritis
and artery
• 2



SOURCE: RAC

Broad focus. The majority of trials now target diseases with large patient populations.

growing emphasis on this disease, says Wivel, is that private investment in gene therapy is increasing, and companies can't justify large R&D expenses unless they can expect to treat large patient populations. Another reason is that these patients often have no alternatives in conventional medicine and are

therefore eligible for experimental therapy. Thirty of the 51 cancer trials are designed to insert into tumor cells a gene expressing a substance such as the lymphokine interleukin-2 (an immune-system signaling molecule), in the expectation that it will stimulate a natural immune attack on the tumor cells. Another 11 studies aim to induce tumor cells to express the herpesvirus protein thymidine kinase, which makes them vulnerable to treatment with the drug gancyclovir. The remaining 10 trials test three other strategies, including four trials that seek to stop cancer by activating tumor suppressor genes. No results have yet been published from these trials. Another fast-growing area is gene therapy for AIDS. Indeed, the majority of patients enrolled in clinical trials approved in the first half of 1995—168 out of a total of 268—are participating in tests of an anti-HIV therapy

sponsored by Viagene Inc. of San Diego. (These include a trial approved only by FDA; private trials need not obtain RAC approval.) Viagene has focused on a succession of strategies in at least four RAC-approved HIV trials. These studies aim to put genes that express HIV proteins into some of a patient's cells, in the hope that the cells will express antigens that will prime the immune system to attack infected cells carrying the same antigens. In addition to Viagene's trials, five others go after HIV with other strategies: They seek to disrupt viral functions by creating decoy molecules to compete with, sequester, or cleave products produced by HIV, or they try to cause HIV-infected cells to express thymidine kinase or other molecules that make them targetable by chemical attack. Clinical results have not yet been published from any of these trials.

These two vector types—retrovirus and adenovirus—account for more than 85% of those used in clinical trials. But leading researchers and a few companies are looking for other vehicles. For example, Joseph Glorioso, director of the gene-therapy program at the University of Pittsburgh, is focusing on herpesvirus. It infects the central nervous system and carries a remarkable "latency gene" that hides it from immune attack. In theory, herpesvirus vector could be used to insert DNA into the nervous system. But it is difficult to manipulate and may have hidden risks. Eventually, Glorioso hopes to seek approval to use it for cancer therapy. R. Jude Samulski, leader of the gene-therapy program at the University of North Carolina, is investigating adeno-associated virus (AAV). It has no known toxicities and replicates only in the presence of a "helper virus," making it look very safe. It may have other advantages, says Samulski: It is simple, and its "life cycle" suggests it may be able to persist and deliver genes for a long time. But Samulski acknowledges a common criticism: AAV is difficult to produce in large quantities. One leading gene-therapy researcher who asked not to be named claims that current AAV technology is inefficient and expensive, adding: "AAV is like an onion—the more layers you peel off, the more you cry." So far, only

one human trial using AAV—for treatment of cystic fibrosis—has been approved; no data are yet available. But Samulski predicts, "You'll see more and more AAV protocols." Viagene Inc. of San Diego is investigating sindbis—an African virus that infects the nervous system—as a new vector. Its virtues include a unique and highly productive method of replication. Other groups are looking into HIV as a vector, although probably only for treating HIV patients. The hottest alternatives to viruses are oily substances known as cationic liposomes. These concoctions, which come in many varieties, can slip DNA into the cell's nucleus and cause genes to be expressed. One skilled user of the technology, Gary Nabel, a Howard Hughes Medical Institute investigator at the University of Michigan, predicts liposomes will improve dramatically in the next few years, increasing levels of gene expression by "an order of magnitude or more," and that they will quickly be adapted for clinical use. In addition to liposomes, two other nonviral vectors have been adopted for clinical trials: direct injection of plasmid DNA into the muscle (two trials) and air-gun injection of a DNA-coated pellet (one trial). These examples should make clear that there is as yet no perfect

VECTORS IN RAC-APPROVED CLINICAL TRIALS			
Vector	Number of clinical trials	Pluses	Minuses
VIRAL			
Retrovirus	76	Efficient transfer Easy to make	Small capacity Random DNA insertion Dividing cells only Replication risk
Adenovirus	15	Nondividing cells Possibly targetable	Immunogenic Replication risk
Adeno-associated virus	1	Nonimmunogenic	Small capacity Hard to make
Herpesvirus	0	Nonimmunogenic Targets CNS	Risks unclear Hard to make
NONVIRAL			
Liposomes	12	No replication Nonimmunogenic	Low efficiency
Plasmid	3	No replication Nonimmunogenic	Low efficiency

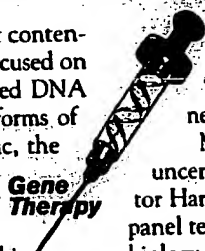
vector. And many researchers say we shouldn't expect one: Instead, there will be a confusing array of viral bits and pieces, combined with other gene-transfer agents, all of them used in custom tools designed for specific applications. But if a perfect vector were to be created, it might look something like the one being pursued by the intramural research staff at NCHGR. NCHGR has launched a project, led by Melissa Rosenfeld and Paul Liu, to develop what they call a "human artificial chromosome." The idea is to create a synthetic 25th chromosome, big enough to transport whole "suites" of genes into the nucleus of a target cell, including all the regulatory sequences that surround a critical gene. Collins told a review panel in May that NCHGR is collaborating with a few extramural groups in an "intense" effort to push this "high-risk" project forward. But a staffer notes that it hasn't yet achieved "proof of principle." For the present, Collins observed, "the paucity of clinically acceptable gene transfer techniques severely limits the potential applications of gene therapy." —E.M.

RAC's Identity Crisis

From the start, gene therapy has been one of the most contentious fields in biomedicine. In the early days, debate focused on safety—on the possibility, for example, that engineered DNA might create novel infectious viruses or trigger new forms of cancer. To minimize such risks and reassure the public, the National Institutes of Health (NIH) beginning in 1980 asked all government-funded gene therapy researchers to submit protocols for prior approval by a public panel. Today, these reviews are carried out by NIH's Recombinant DNA Advisory Committee (RAC), a mixed group of 20 scientists and nonscientists who meet quarterly at NIH. RAC has voted on virtually every gene therapy trial in the United States.

Now, after 5 years of clinical experimentation and no evidence that gene therapy poses a general risk to the public, fears are fading and, with them, the justification for RAC. Some leaders in gene therapy—especially researchers eager to get experiments launched and companies with large sums hanging on clinical trials—are saying it's time for RAC to think about retiring. They point out that the law already requires the Food and Drug Administration (FDA) to monitor clinical trials and clear therapeutic products for safety and efficacy, which means that gene therapy has to pass two federal checkpoints.

Stephen Marcus, an executive at Genetic Therapy Inc. of Gaithersburg, Maryland, says, for example, that RAC delays by 2 months or more decisions ultimately made at the FDA. "It may be a little hyperbolic" to suggest that lives are being lost as a result, Marcus says, "but the concept is there." Likewise, Thomas Reynolds



of Targeted Genetics in Seattle complains that RAC has become a "bottleneck." He'd rather see it focus on "novel issues, like germ-line therapy, new vector systems, new disease targets"—not on reviews of individual protocols.

Members of the RAC themselves have also expressed uncertainty about the role they're expected to play, NIH Director Harold Varmus has observed. While public members of the panel tend to focus on safety and ethics, those with expertise in biology often zero in on technical aspects of proposals that they find inadequate. RAC has thus suffered from a split personality. To clarify the committee's proper role, Varmus has taken a couple of steps in the past year. First, he has asked RAC's staff and the FDA to work out a unified review process, now being put into effect, that may allow researchers to submit a single application for review by both agencies. Only those that raise new technical or ethical issues would be debated by the RAC.

Second, Varmus has appointed a special ad hoc study group—headed by oncogene researcher Inder Verma of the Salk Institute in La Jolla, California—to consider how NIH should review gene therapy in the future. One of the big questions to be addressed is: Who, if anyone, should scrutinize clinical trials for scientific value? Officially, that isn't RAC's job, although expert members find it hard not to comment on technical quality. The Verma panel will deliver its recommendations on this and other broad questions about how NIH should handle gene therapy trials to Varmus by December.

—E.M.

Most of the other trials reviewed by RAC are not aimed at delivering therapy: They are designed to tag specific cells with genetic markers to provide information about the fate of the cells. When RAC members sifted through the catalog of these "gene marking" trials in June, they found that although this area gets little public attention, it is in fact scientifically the most encouraging area. Smith says they have produced at least four peer-reviewed publications laden with "hard data." The research has shown, for example, that cancer relapse following autologous bone marrow transplants—in which a patient's bone marrow is removed before intensive chemotherapy and later replaced—often is caused by tumor cells that survive in the marrow. It indicates that clinical research should zero in on ways to purge tumor cells from the marrow.

Whether this overall picture is judged positive depends in large measure on who's being asked. Pioneer gene therapists and industry leaders tend to view the explosion of trials as evidence of progress. Independent academics, on the other hand, often see the glass as

half empty. But both sides can agree that, at the least, the field isn't harming its patients. Clinical trials, says Smith, have shown few signs of toxicity and no hints of runaway genetic mutations: "There are no three-headed cows" of the kind anticipated in *National Enquirer*-land," he jokes. But the disappointing news, Smith finds, is that so far only hints of therapeutic benefit have appeared.

Wivel notes that nearly all the gene therapy trials so far have been "phase I" trials, designed to test safety rather than efficacy. So they can't really be judged on effectiveness. But that hasn't discouraged some gene therapy leaders from trying. Stephen Marcus, a vice president at Genetic Therapy Inc. of Gaithersburg, Maryland, cites a brain cancer patient who, after surgery for glioblastoma, was treated with GTI's anti-cancer gene therapy and has survived for more than 2 years. This is almost unheard of, Marcus says, and is clearly "a case where there is some evidence of effectiveness." He notes, however, that "we realize this is anecdotal."

But the RAC members who reviewed cancer trials—Robert Erickson of the

University of Arizona and R. Jude Samulski of the University of North Carolina—deemed it "too early" to reach any conclusion. Erickson found several unpublished reports that gene therapy had reduced tumor size, but noted that other, simpler therapies have produced similar reports in the past. Samulski pointed to a common theme running through the cancer studies that raised some concern: low rates of gene transfer.

Indeed, difficulties in getting genes transferred efficiently to target cells—and getting them expressed—remain a nagging problem for the entire field. Virus-based vectors have been the most efficient for inserting genes into cells in the lab, but they have run into problems in the clinic. Often the fraction of cells receiving the new gene is low, particularly if these targets of gene therapy are long-lived "stem" cells that give birth to other cells. Researchers say it has been difficult to achieve a 1% rate of gene transfer into stem cells, for reasons not fully understood. And even when genes are inserted in stem cells, they may not be active in second-generation cells, yielding less-than-adequate therapy.

Boosting the rate of gene transfer by increasing the concentration of vector or by dosing patients repeatedly may create another problem, however: It may stimulate the immune system to attack and neutralize the therapy-bearing cells. Francis Collins, director of NIH's National Center for Hu-



Back to basics. James Wilson wants less hype, more research.

VICKI VALERIO/THE PHILADELPHIA INQUIRER

man Genome Research, told the Motulsky-Orkin panel in May that "many problems must be solved before gene therapy will be useful for more than the rare application."

Voting with their checkbooks

Academic researchers are still grappling with many fundamental issues in gene therapy. But industry leaders and their financial agents are gung-ho. Investors have poured hundreds of millions of dollars over the past 5 years into gene-therapy companies, drawn by hopes of blockbuster discoveries. And big companies are now getting into the act. Late last year, Switzerland's Ciba-Geigy Ltd. acquired a 49.5% share of Chiron Corp. of Emeryville, California, which then turned around in April 1995 and began buying Viagene. Less than 3 months later, another Swiss pharmaceutical giant, Sandoz AG, bought GTI—an investment that gives Sandoz rights to GTI's broad patent for "ex vivo" therapy, in which cells are removed from the patient, given new genes, and replaced (Science, 31 March, p. 1899). Also last fall, the French company Rhône-Poulenc Rorer struck agreements with a network of small companies to gain access to the latest research (Science, 18 November 1994, p. 1151).

One result of this burgeoning investment is that private companies have come to dominate clinical trials of experimental gene therapies. By June, according to the RAC, 13 firms had won approval to run at least 34 gene-therapy trials—so that now, 60% of all therapeutic trials are privately funded. Industry also plays an indirect role in physician-sponsored trials, supplying vectors at little or no cost.

This trend is worrying some leaders in the field, who say biotech companies are forcing the pace and direction of research, and not always in ways anchored in the best science. Varmus, for example, says that while it's "a good thing" that investors are willing to pick up the tab for "very expensive" clinical experiments, these trials absorb "a lot of resources and talent," and he isn't sure that they "are scientifically as worthy as other things that could be done." He's concerned about understanding the biology of viruses used to transfer genes and of the immune reactions they provoke.

Varmus isn't alone in expressing concerns. James Wilson, director of the Institute for Gene Therapy at the University of Pennsylvania, says private funding is important, but he worries that expectations may be raised pre-

maturely. People who invest in gene therapy anticipate a big payoff, but they may not realize how long it will take, Wilson says. "The actual vectors—how we're going to practice our trade—haven't been discovered" yet, he notes, "so it may be early for the impatience of venture capital-supported biotech."

This commercial pressure may also account for some of the hype surrounding developments in gene therapy, says Wilson. If you're the leader of a gene-therapy company, "you try to put as positive a spin as you possibly can" on every step of the research process, he notes, "because you have to create promise out of what you have—that's your value." But, Wilson says, "that's not what we need right now." What the field needs is "a lot of basic research on vectors and cell biology."

Pardoll of Hopkins is equally critical; he says that in the rush to get trials approved, "biological principles are not well thought out—especially immunological principles." Varmus says this happens because the main

calls it "the patent from hell" because it's so broad. He thinks it may discourage newcomers and stifle collaboration. When Miller made this comment at a RAC meeting in June, GTI President James Barrett rose to say the company considers the patent "valid" and will negotiate reasonable terms that are "idiosyncratic" for each use.

Academic scientists may think it's too early to be talking about financial returns, but not company executives and some industry analysts. Take Wall Street biotech analyst Jeffrey Swarz of the investment bank CS First Boston. Swarz delivered an enthusiastic assessment of the field at last year's congressional hearings and was equally bullish in a recent interview with Science. Gene therapy for cystic fibrosis, he said, "has been successful; ADA disease has been successful; brain cancer has been successful. ... So far, the technology looks fabulous." He predicts a gene-therapy product will reach the market by next year.

At a recent meeting in Washington, D.C., organized by the Institute for Interna-

tional Research, gene-therapy business chiefs were asked when they thought their industry's first product would hit the market. Few were as optimistic as Swarz, but the forecasts ranged from very soon—in 1997, according to David Nance, president of Introgen Therapeutics of Austin, Texas—to reasonably soon—in 2000, according to Harvey Berger, chair of Ariad Pharmaceuticals in Cambridge, Massachusetts. One attendee, Mark Edwards, managing director of Recombinant Capital, an independent San Francisco firm that analyzes biotech companies, was less ebullient, saying he didn't expect a

commercial product until 2003. Whether one's an optimist or not, concluded Berger, "we've got to make sure the biology matches the enthusiasm."

Many academic gene therapists agree with Berger, and some have said they hope the critical review Varmus has ordered from the Motulsky-Orkin panel will cut through the hype that surrounds the field and inform the public that it could be many years before the money invested in clinical trials yields a product. "It may be time for some realism," says Michael Knowles of the University of North Carolina's cystic fibrosis program. Adds Joe Glorioso, director of the University of Pittsburgh's gene-therapy program: "We just can't be wimpy about this: we have to be in for the long haul."

—Eliot Marshall

U.S. GENE THERAPY TRIALS SPONSORED BY INDUSTRY

Company Sponsor	Founded	Capital (\$millions)	No. of Trials	Topic
Applied Immune Sciences	1982	209	1	Cancer
Canji	1990	21	1	Cancer
Cell Genesys	1988	103	1	HIV
GenVec	1992	20	1	Cystic fibrosis
GeneMedicine	1992	50	1	Alpha-1 anti-trypsin
Genetic Therapy	1986	103	6	Cancer, CF, Gaucher
Genzyme	1981	74	5	Cancer, CF, Gaucher
Immune Response	1986	128	1	Cancer
Ingenex	1992	5	2	Cancer
Introgen Therapeutics	1993	NA	3	Cancer
Somatix Therapy	1979	102	3	Cancer
Targeted Genetics	1989	46	1	HIV
Viagene	1987	106	6	Cancer, HIV
Vical	1987	46	4	Cancer, HIV

SOURCE: COMPANY DATA AND RAC

concern of small companies is to survive, and "one way to survive is to have a clinical trial—show that you're actually on the scoreboard." But promoting the company doesn't necessarily promote gene therapy, Varmus notes: "We're not talking about an industry that's in an advanced state of competence."

Another effect of commercial investment, some researchers say, has been to channel energy into intellectual property disputes and turf battles. For example, Dusty Miller, a virologist at the Fred Hutchinson Cancer Research Center in Seattle, argues that the gene-therapy patent issued to NIH and GTI in April will have a "chilling effect" on research. The patent covers all forms of ex vivo therapy. Miller—who was among those involved in the research that led to this patent but was not named as a co-inventor—

Targeted vectors for gene therapy

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ABSTRACT Successful gene therapy requires not only the identification of an appropriate therapeutic gene for treatment of the disease, but also a delivery system by which that gene can be delivered to the desired cell type both efficiently and accurately. Reductions in accuracy will inevitably also reduce efficiency since fewer particles will be available for delivery to the correct cells if many are sequestered into nontarget cells. In addition, the therapy will have net benefit to the patient only if gene delivery is sufficiently restricted such that normal cells are left unaffected by any detrimental effects of bystander cell transduction. Here we review how currently available delivery systems, both plasmid and viral, can be manipulated to improve their targeting to specific cell types. Currently, targeting is achieved by engineering of the surface components of viruses and liposomes to achieve discrimination at the level of target cell recognition and/or by incorporating transcriptional elements into plasmid or viral genomes such that the therapeutic gene is expressed only in certain target cell types. In addition, we discuss emerging vectors and suggest how gene therapy delivery systems of the future will be composites of the best features of diverse vectors already in use. — Miller, N., Vile, R. Targeted vectors for gene therapy. *FASEB J.* 9, 190-199 (1995)

Key Words: targeting • retrovirus • adenovirus • liposome

THE IDENTIFICATION OF THE UNDERLYING genetic defects has recently made gene therapy an attractive treatment option for a wide variety of diseases. However, there is a corresponding requirement to produce vector systems that can deliver therapeutic genes to the appropriate target cells either *in vivo* or *ex vivo*. These systems must be both *efficient* and *accurate*. The range of different diseases amenable to intervention by gene therapy means, however, that no single delivery system is likely to be universally appropriate. For instance, the requirements of gene therapy for cystic fibrosis are greatly different from those of cancer. In the former case, only a certain proportion of a localized population of cells needs to be targeted with a single corrective gene; by contrast, cancer gene therapy usually involves the targeting of all of a diffusely spread population of cells, with the ultimate aim of killing rather than correcting them. Hence, the stringency with which the therapeutic gene needs to be accurately delivered can vary greatly. Expression of a copy of the cystic fibrosis transporter gene in nontarget cells is likely to be much less toxic than inadvertent expression of cytotoxic genes, aimed at cancer cells, but expressed in normal bystander cells.

Here, we review the progress in targeting gene delivery systems to specific target cell populations and look forward to the areas of research that will bring developments for the future. Unfortunately, improvements in the accuracy of a

vector often compromise its efficiency, and vice versa. Nonetheless, it is clear that the technology now exists to incorporate specific targeting features into most of the currently available delivery systems. These may be at the level of 1) target cell surface recognition, by manipulating the surface recognition components of viruses and liposomes; or 2) target cell transcriptional restrictions, by incorporating transcriptional elements into plasmid or viral genomes such that the therapeutic gene is expressed only in certain target cell types.

The ultimate aim for the vectors of the future is to include these and other targeting opportunities within the same vehicle. In all probability, this will involve the incorporation of the most beneficial features of a variety of viral and nonviral systems into a single hybrid vector specifically custom built for each individual therapeutic situation.

TARGETING OF GENE THERAPY VECTORS AT THE LEVEL OF THE CELL SURFACE

Retroviral vectors

A primary determinant of retrovirus infectivity is the interaction between specific receptors on the host cell surface and glycoproteins (Env) on the lipid envelope of the retroviral particle. Ideally, targeted retroviral vectors for human gene therapy would use safe recombinant genomes and packaging lines from wild-type retroviruses that naturally display envelope proteins with the required tropisms. However, few naturally occurring retroviral infections are strictly limited to one cell type (1), and of the known receptors for retroviruses, only the HIV-1/SIV receptor CD-4 (2) is of relatively restricted distribution. Attempts have been made to produce vectors and packaging lines from HIV (3). However, HIV is a complex retrovirus that requires a number of self-encoded autoregulatory proteins, and this complicates the construction of stable packaging lines. Nevertheless, the principle of a recombinant HIV genome as a gene vector for CD4⁺ cells has been demonstrated (3). However, vectors carrying HIV-1 *env* sequences would have to be used with extreme caution as the HIV-Env protein itself may be neurotoxic (4) or even immunosuppressive.

Most recombinant retroviral vectors and packaging lines produced so far have been based on murine leukemia viruses (MLVs)² (5). There are five recognized MLV groups (1) as

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²Abbreviations: MLVs, murine leukemia viruses; MLV-E, ecotropic strain of MLV; MLV-A, amphotropic strain of MLV; RES, reticuloendothelial system; PEG, polyethylene glycol; ReSV, respiratory syncytial virus; ASOR, asialoorosomucoid; LCRs, locus control regions; DTA, diphtheria toxin A; MVM, mouse minute virus.

defined by tropism, of which the most useful for gene delivery purposes have been the ecotropic strain (MLV-E), which infects virtually all rodent cells, and the amphotropic strain (MLV-A), which infects practically all mammalian cells. Packaging lines have therefore been created to allow production of retroviral vectors with host ranges that are either ecotropic or amphotropic, respectively (5). It is likely that all retroviral vectors suitable for human gene therapy in the near future will be based on such recombinant MLV genomes because they are well characterized with regard to safety and efficiency. For targeted retroviral vectors, then, the problem is either to restrict the promiscuous tropism of amphotropic particles or to confer upon ecotropic particles a limited human cell affinity. This could be done either by: 1) genetic manipulation of the producer line such that amphotropic or ecotropic Env is replaced by a different viral or nonviral protein having the required affinity; 2) directly engineering a particular affinity into Env; or 3) molecular conjugate approaches, in which ligands are coupled to the outside of the retroviral particle.

Replacement of Env: retroviral pseudotypes

The facility (5) with which *trans*- and *cis*-acting functions can be separated in MLV packaging lines allows easy experimental manipulation of the *trans*-acting function responsible for cellular tropism, namely, Env. This raises the possibility of replacing one viral *env* with that of another, thereby creating a hybrid producer line that generates "pseudotyped" viral vectors with a tropism conferred by the replacement *env* (Fig. 1). Phenotypic mixing has been used for many years as a tool to study receptor interactions (see ref 1 for a review); however, efforts have recently been directed at precisely replacing *env* and producing not envelope mixtures but vector populations exclusively displaying a novel tropism (1, 6). Such hybrid formation in general seems to occur more

efficiently between closely related viruses. For instance, a recombinant MoMLV genome can be rescued by C-type viruses but not by HTLV-I or D-type viruses (7). However, provision of homologous or more closely related Gag proteins in some cases relaxes phenotypic restrictions on efficient pseudotyping of vector genomes with exogenous Env; for instance, an MoMLV vector can be packaged inside HTLV-I (8) envelopes when MoMLV *gag-pol* are supplied *trans*. Similarly, HIV has been given an extended host cell range by pseudotyping with the unrelated viruses HSV and VSV (9). Although these examples demonstrate the principle of creating an improved retroviral vector for human gene therapy by pseudotyping, so far they have produced only vectors with extended tropism rather than with restricted specificities.

The logical and necessary extension of pseudotyping approaches, then is to replace retroviral envelope genes with genes derived from nonviral sources. Although there are instances of nonviral glycoproteins being preferentially incorporated into retroviral particles, such as Thy-1 (10) and CD4 (11), actual infection of target cells, as opposed to specific binding, via display of such nonviral proteins has not been demonstrated, and is likely to require either fusogenic sequences within the foreign protein itself or coexpression of fusogenic molecules on the viral envelope.

Engineering Env

Genetic manipulations whereby sequences conferring specific binding affinities are engineered into preexisting viral *env* genes represent a promising approach. In MoMLV the sequences that determine receptor specificity seem to be in the most distal of the two variable regions within the amino-terminal portion of the SU Env subunit, and replacement of the variable region of one strain with that of another can, for instance, change viral tropism from that of strain

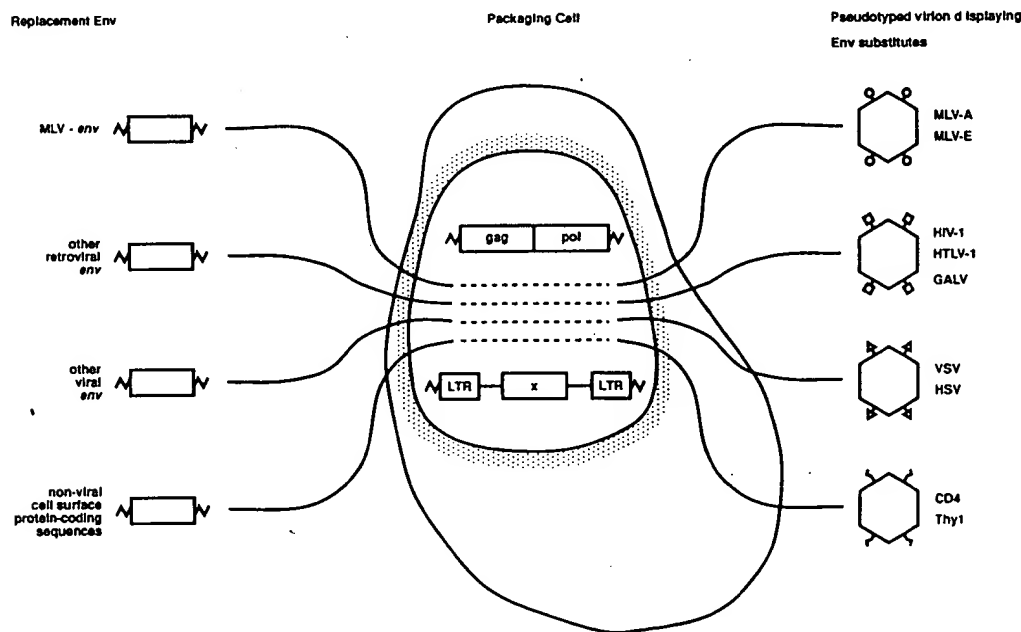


Figure 1. Generation of retroviral vectors with novel tropisms by construction of hybrid packaging lines. Transfection of a cell with genes (*gag-pol*, *env*) that encode viral *trans*-acting functions allows expression of all the structural components of the virion by that cell; these components can recognize and package the recombinant retroviral genome (shown here bounded by long terminal repeats (LTRs) and carrying a therapeutic gene *x*). Here we represent diagrammatically the various classes of retroviral pseudotypes that have been produced by providing various *env* genes *trans*; this illustrates the principle of alteration of retroviral vector tropism by pseudotyping.

4070A to that of 10A1 (12). Engineering of murine retroviral Env proteins is being actively investigated (12-14) and is an important area of research. However, receptor recognition may involve complex interactions between the cellular ligand and different parts of the viral Env, and modification of viral tropism by direct replacement of receptor-binding sequences will not be straightforward. The function of Env proteins is not simply to adhere to host cells but also to participate in a sequence of events leading to membrane fusion. Excessive alteration of Env structure might therefore jeopardize the exposure of hydrophobic domains required for fusion and correct viral internalization. Nevertheless, a mammalian cell tropism has been conferred on an avian retrovirus by engineering integrin-binding sequences into Env. It was found that two of the variable regions of ALV Env could be manipulated by exchanging *env* sequences with those encoding a 16-amino acid RGD-containing peptide to produce Env proteins that were processed and incorporated into retroviral particles (15). Such hybrid envelopes could still efficiently mediate infection of avian cells through the ALV receptor, and could also infect and transfer neomycin resistance to mammalian (ALV-refractory) cells that expressed RGD-recognizing integrins. Infection was not efficient and required previous deglycosylation of the virus to expose RGD epitopes, but it is an important demonstration of the principle of targeting retroviral vectors by envelope modification.

In other studies, the RSV host range has been broadened to include human cells by packaging the genome with a chimeric Env that was a fusion of the RSV signal peptide and the influenza virus hemagglutinin (16). Chimeric Env was found to be incorporated into the virions as efficiently as wild-type RSV Env. It may be possible to use influenza hemagglutinins to direct retroviral vectors to subsets of cells exhibiting particular glycosylation phenotypes as the various influenza strains possess different hemagglutinins with different precise specificities. Another candidate protein for restriction of tropism is the B19 parvovirus surface protein, the surface receptor for which has recently been characterized (17) as the tetrasaccharide of globoside (blood group P antigen), which has a very limited tissue distribution. The B19 surface protein may be susceptible to fine-tuning of saccharide specificity by recombinant techniques or site-directed mutagenesis, similar to the influenza hemagglutinin (18).

The possibility of targeting retroviral vectors to particular glycosylation phenotypes may be of special interest for cancer therapy, as many transformed cells show altered glycosylation. Whether or not any aberrantly expressed glycans can mediate viral entry is another question; a recent report indicates that retroviruses targeted to cells via lectin cross-linking cannot infect the cells after binding (19), but this could be a function of the lectin or of structural alterations caused by cross-linking rather than a function of the glycan receptor.

The demonstrable ability (16) to alter RSV tropism from avian to human cells by manipulation of envelope structure could be of great interest for cancer therapy. This is because the vast number of target cells in malignant disease suggests that either the immune system must be recruited or that a replicating vector be used to target all the tumor cells, and RSV is a replicating vector par excellence. Besides its own genome, this virus is known to carry a cell-derived oncogene; replacement of this with a therapeutic cDNA would give a replication-competent gene therapy vector.

Encouraging results have been reported using a similar approach, in which a cDNA encoding an mAb fragment

capable of hapten recognition was fused to the *env* gene of MoMLV (18). Coexpression of this gene with the normal envelope in an ecotropic packaging line resulted in infective viral particles that possessed the appropriate hapten-binding activity. It should be noted that the packaging line was expressing and required parental ecotropic Env as well as the chimeric protein, so it remains to be seen if infective retroviral particles can be assembled that contain only hapten-displaying Env (20). This approach has yet to be demonstrated using a hapten directed against a relevant human antigen capable of mediating virus internalization, and is still far from in vivo application.

Targeting by retrovirus-ligand conjugates

Hepatocytes possess a unique receptor that internalizes asialoglycoproteins. Conjugation of lactose to ecotropic viral particles allowed them to be recognized as asialoglycoproteins and broadened their host range to include human hepatoma cells (21). However, this approach is limited first to cells that express the asialoglycoprotein receptor, and second to proliferating cells (because retroviruses depend on host cell mitosis in order to integrate). As normal liver cells have a very low turnover rate, this technique is most likely to be of use for in vivo delivery to malignant liver disease of the hepatocyte lineage. Furthermore, because the vector was based on an ecotropic virus, its tropism in humans would be limited entirely to hepatocytes, greatly increasing its safety compared with broad affinity vectors such as those bearing the 4070A or GALV envelope proteins.

In a more indirect approach, it was found that ecotropic MoMLV vectors bound to human hepatoma cells after being cross-linked to the transferrin receptor by a series of antibodies; however, there was no subsequent proviral integration, suggesting either that the cross-linking antibodies were inhibiting membrane fusion or that the transferrin receptor cannot mediate appropriate viral internalization (22). A similar cross-linked mAb technique has been used to target ecotropic retroviral particles to human cells in vitro by means of the streptavidin-biotin reaction (23). This allowed ecotropic virus to bind to cells expressing human class I or II MHC antigens and to become internalized and integrated. An extension of this technique (19) showed that biotinylated EGF or insulin could substitute for the anticellular receptor antibody, and that EGF and insulin receptors could mediate internalization, leading to integration, of retroviral particles bearing streptavidin-conjugated antibodies. The possibility of targeting retroviral vectors by means other than murine antibodies, which suffer from numerous disadvantages in vivo, suggests that this approach may have potential although its in vivo applicability has yet to be demonstrated.

Adenoviral vectors

Adenoviruses are double-stranded DNA viruses in which the viral genomic DNA is contained in a virally encoded protein coat (capsid) rather than a phospholipid bilayer of host cell origin. The capsid consists of three major types of subunit: the hexon, which makes up the bulk of the coat; the penton base; and the penton fiber. The fiber is attached to the capsid via the penton base and projects outward; base and fiber together are known as the penton complex. During infection, the fiber mediates initial binding of the virus to an unidentified cellular receptor and the penton base subsequently mediates virus internalization via interactions with α_v -type integrins (24). Thus, the penton complex is respon-

sible for binding and internalization, and therefore for viral tropism at the level of cell recognition. Although adenoviral diseases are usually associated in vivo with respiratory epithelium or the GI tract, their cellular receptors seem to be widely distributed (25). Clearly then, as with retroviruses, the problem is to limit viral tropism to a particular subset of tissues. The adenoviral proteins responsible for attachment and internalization, respectively, have been well characterized, giving two points at which to manipulate tropism. The most promising approach is to restrict adenovirus infection at the cell-binding stage by replacing the carboxyl-terminus knob of the fiber with a ligand conferring a particular tropism, for instance, with an antibody hapten. One report (26) describes the restriction of adenovirus type 5 tropism by a different kind of fiber modification where intact virions were chemically modified so that their fiber carbohydrate groups were covalently linked to an asialoglycoprotein-polylysine conjugate. Such modified virus was found to have much decreased infectivity to asialoglycoprotein receptor-negative cells while retaining infectivity to receptor-positive cells. This approach would be equally applicable to targeting adenoviral vectors per se. It may also be possible to restrict infection by replacing the RGD-containing domain of the penton base with sequences having affinity for a ligand other than RGD-recognizing integrins.

Adenoviral vectors can also be targeted via the route of administration (27); targeting of a *lacZ*-expressing adenoviral vector to the kidney by renal artery or pyelic cavity infusion resulted in β -gal activity in various renal cells with no detectable expression in liver, lung, or bladder cells (27).

A possible advantage of refinement of vector targeting to the point of absolute specificity might be the ability to use replicating vectors for gene therapy. For cancer, development of a replicating adenoviral vector, perhaps carrying a cytokine or suicide gene, targeted to cancer cells at the level of cell binding (via fiber/base manipulations) and at the level of transcription (see next section) might allow transduction of the large number of malignant cells in a tumor deposit; cell death due to adenovirally induced lysis may even potentiate the field effect of cytokines. A safety feature of such a system would be that the immune system would be expected to eventually clear such therapeutic infections (as it does for wild-type infections); therefore this potential therapy only awaits adequate targeting strategies.

Liposome vectors

Most work on targeted liposomes has been designed to deliver cytotoxic drugs to cancer cells and has been reviewed recently (28). Expression of a cDNA in the target cells makes greater demands on the vector system in that it must not only target the appropriate cell type but also allow efficient delivery of undegraded DNA to the nucleus. For most targeted gene delivery purposes, conventional liposomes are limited because of their selective uptake by cells of the reticuloendothelial system (RES), in particular by macrophages resident in liver, spleen, and bone marrow, because of their limited extent of extravasation. Where macrophages themselves are the target, however, RES affinity is advantageous. In *L. donovani* leishmaniasis parasites not only multiply in the Kupffer cells of the liver, but are also resident in a vacuole to which lysosomes fuse, so that liposomes are passively targeted not only to the parasitized cell but also to the appropriate organelle, making liposome-mediated delivery of transcriptionally targeted antisense or suicide genes to these parasites a real possibility. It is also possible in a few cases to avoid much of the RES by the particular route of ap-

plication, particularly where the target tissue is found in a discrete anatomical compartment; e.g., nontargeted liposomes could be applied directly to the bladder for treatment of carcinoma or to the lung for treatment of cystic fibrosis or α AT deficiency. Targeting by compartment has allowed confined transduction of discrete sections of arterial wall using both liposomal and retroviral vectors (29).

In most cases, however, in vivo use of liposomes requires first avoiding the RES, and second, display of appropriate tropic and fusogenic molecules (Fig. 2). Uptake by the RES can be considerably delayed, but not altogether avoided, by the use of "stealth" liposomes that display negatively charged moieties such as the ganglioside GM1 and polyethylene glycol (PEG) (28). For most systemic purposes, the stealth formula is probably essential.

Liposomes bearing an immunoglobulin complement ("immunoliposomes") can exhibit tropisms conferred by the displayed antibody. Hence, coupling to liposomes of an antibody against glioma cells increased the efficiency of gene delivery to these cells in culture by about sevenfold (30). Just as mAbs may be conjugated to liposomes to confer targeting capability, so may other ligands such as growth factors and hormones. Coupling of transferrin to liposomes followed by i.v. injection in a rabbit model resulted in significantly greater localization to bone marrow erythroblasts (31), and incorporation of surfactant protein A into liposomes increased the uptake of the liposome cargo by alveolar type II cells (32). However, it is not sufficient merely to confer upon the vector a particular binding ability; the particle must bind to a ligand that also allows fusion of liposome and cell membranes. Such considerations of appropriate internalization of vector cargo are especially important for gene delivery vectors, where the DNA must not only reach the appropriate cell type but also must reach the nucleus in undegraded form.

Conjugating virions to liposomes or incorporating viral surface glycoproteins into liposomes might create a vector that has the efficient cell attachment and entry mechanisms of a virus but not the safety drawbacks; much work has been done in this area with Sendai virus in particular (33). Another system used liposomes that displayed only the fusogenic protein of Sendai virus (F-protein) and not the cell-binding protein (hemagglutinin) (34). However, although

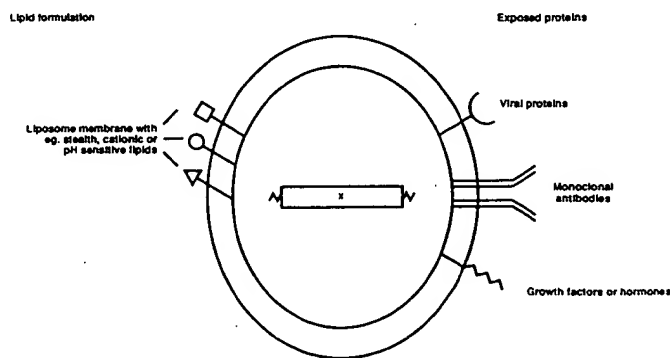


Figure 2. Modification of lipid membranes to produce targeted liposomes. Targeting of liposomes requires first abrogation of their RES affinity, and second, provision with exposed ligands having the required targeting capacity. Inclusion of ganglioside glycolipids into the lipid formulation can allow RES evasion; other lipid formulations include cationic lipids to allow promiscuous membrane binding and hence lysosome escape, and pH-sensitive lipids, which allow lysosome escape without the broad affinity conferred by cationic lipids. Various types of ligand can be inserted into the lipid membrane for provision of particular tropisms (see text for details).

such approaches can make liposomes up to 10-fold more efficient than lipofection at gene delivery (33), in terms of targeting all it can do is confer upon the liposome the tropism of the virus, and there are very few native viral receptors that exhibit a narrow and precise cell type specificity. Nevertheless, a promising system (35) is currently being developed in which respiratory epithelium is targeted by means of the surface proteins of respiratory syncytial virus (ReSV), which is responsible for infections of the lower respiratory tract. Liposome-type envelopes were constructed that displayed both the attachment and fusion proteins of ReSV, and these have been shown to enter all cells of a cultured respiratory epithelial cell line within 1 h (35).

Cationic liposomes such as the commercially produced lipofectin can efficiently avoid the lysosomal pathway because the particular lipid composition allows direct fusion of liposome and cell membranes. These particles are therefore much more efficient than conventional liposomes, and for *in vitro* transduction have largely replaced them. Cationic liposomes have also been used for *in vivo* approaches and even clinical trials; however, there seem to be no data on the extent to which these liposomes can avoid the RES, and indeed the cationic surface would seem to be incompatible with the negative charges characteristic of the stealth formulation. One report suggests that the cationic liposome has as much affinity for other cell types as for the RES after *i.v.* injection (36). Administration of liposomes carrying SV40-CAT resulted in widespread expression of the marker gene for up to 9 wk, albeit mainly in tissues generally associated with the RES such as spleen, liver, lymph nodes, and bone marrow as well as in vascular endothelium. CAT expression was also observed in tumor cells in this experiment, probably as a

consequence of leaky tumor vasculature. It may eventually be possible to combine the efficient lysosomal avoidance of cationic liposomes with a specific targeting capacity, although the problem is likely to be that the generally fusogenic nature of cationic liposomes may preclude any precisely restricted targeting.

Molecular conjugate vectors

Targeting of plasmid DNA may be achieved by coupling the DNA to a ligand with a demonstrated cell or tissue affinity. This is usually brought about by covalently linking a polycation such as polylysine to the ligand; the polycation can then bind to and condense plasmid DNA via electrostatic interactions, leaving the ligand exposed on the surface of the conjugate (37). The ligands chosen must be efficiently endocytosed in the target cells so that DNA is efficiently internalized. One of the first receptors to be used in this way was the asialoglycoprotein receptor, whose expression is limited to hepatocytes; this receptor binds glycoproteins with terminal galactose residues for removal from the circulation; asialoorosomucoid (ASOR) is a major natural ligand for this receptor. BSA has been given specificity for the ASOR receptor by artificial galactosylation, and has been used to target CAT and human factor IX cDNAs (38) to hepatoma cells *in vitro* and to liver but not other tissues *in vivo*. Other ligands that have been used in similar conjugates include insulin (39), EGF (40), lectins (41), and transferrin (37). A major drawback of classical molecular conjugate vectors is that internalization depends on receptor-mediated endocytosis, a process that directs the receptor complex to lysosomes where it is degraded; only a small fraction of introduced

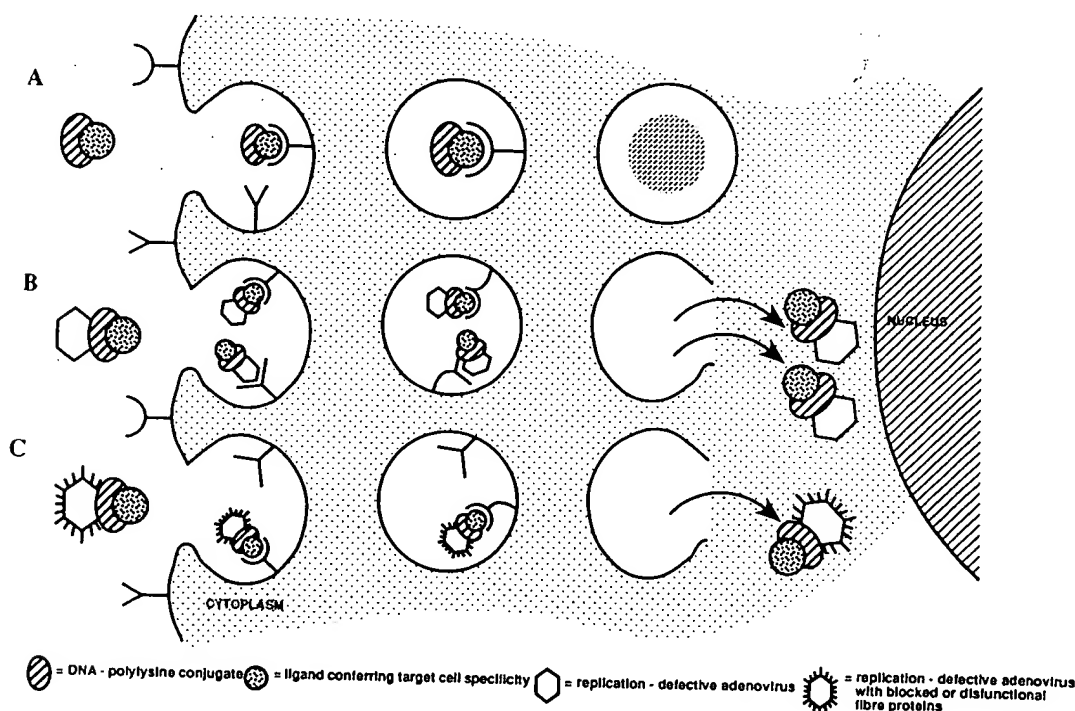


Figure 3. Targeting of plasmid DNA by molecular conjugate vectors. Conjugation of plasmid DNA to a particular ligand can confer a particular targeting capacity, but results in a vector of very low efficiency because most receptor-mediated endocytosis directs such conjugates to lysosomes where the great majority of vector DNA is degraded (route A). By complexing an adenovirus coat to the conjugate, a highly efficient vector is created by virtue of the ability of adenovirus proteins to disrupt the endosome before vector degradation (route B); however, this abrogates any targeting capacity conferred by the ligand, as the complex can enter cells either via the ligand receptor or via the virtually ubiquitous adenoviral receptor. To truly target such complexes it will be necessary to use modified adenoviral coats that retain the lysosomal escape mechanism but cannot interact with the adenoviral receptor (route C).

DNA escapes this pathway and enters the nucleus, leading to low efficiency of transduction.

A new generation of molecular conjugate vectors has been produced that has the capacity to escape the degradative lysosomal pathway by utilizing features of the adenovirus capsid (Fig. 3). Adenovirus disrupts endosomes during cell entry as a consequence of a conformational change in the capsid proteins, resulting in membrane breakdown, triggered by a drop in pH. Hence, molecular conjugate vectors delivered DNA to cells with greatly increased efficiency when transfection was done in the presence of adenovirus. However, this effect relies on both virus and vector being present in the same endosome. To improve efficiency, the adenovirus has been coupled directly to the molecular conjugate (37). However, adenovirus receptors are virtually ubiquitous and so the coupling of an adenovirus receptor to a targeted molecular conjugate would be expected to partially or completely abrogate any preferential tropism conferred by the ligand. Blocking the interaction of fiber with adenovirus receptor by mAb to the fiber resulted (42) in a vector that was both targeted to a specific subset of cells and able to escape the lysosomal pathway. A more satisfactory approach would be to create recombinant adenoviral vectors that display dysfunctional fiber proteins in order to bypass the antibody-coating step.

Few *in vivo* experiments have been attempted using adenovirus-molecular conjugate complexes, and in fact it is unlikely that such vectors will be routinely applicable to *in vivo* work, although they are likely to be of use for *ex vivo* strategies (43). This is a consequence first of the size of the complex (transferrin-polycation conjugates are approximately 100 nm in diameter (44); complexed with AdV they would be even larger), which will prohibit extensive extravasation or tissue penetration, and second, of the likelihood of direct immunogenicity of the AdV proteins (45).

TARGETING OF GENE THERAPY VECTORS AT THE GENETIC LEVEL

Transcriptional targeting

Therapeutic cDNAs may be limited in expression to a particular subset of cells by placing them under the control of regulatory elements that possess binding sites for tissue-restricted positive or negative *trans*-acting factors (Fig. 4). Correctly regulated expression may require, in addition to 5' promoter sequences, distant elements either 5' or 3' to the coding region; these elements act together with the promoter and allow tissue-specific expression at appropriate levels independent of position of integration. Such locus control regions (LCRs) have been identified for a number of genes. LCRs would be of much use for gene augmentation but the transfer of such large sections of DNA to target cells will be problematic, particularly *in vivo*, and in fact for the foreseeable future may be confined to *ex vivo* strategies. Where a monogenic defect results in pathology in more than one tissue, the most pragmatic approach to appropriately limit the expression of therapeutic cDNA is to use the cellular promoter/enhancer elements native to the defective gene. Furthermore, the use of cellular rather than viral promoters reduces the chance of loss of cDNA expression due to inactivation of viral sequences by methylation or other mechanisms (46). Thus, cellular promoters may confer benefits both of long-term expression and of tissue-restricted expression, and where vector-targeting at the cell-binding level has not been achieved it may represent the only way of limiting expression of exogenous cDNA.

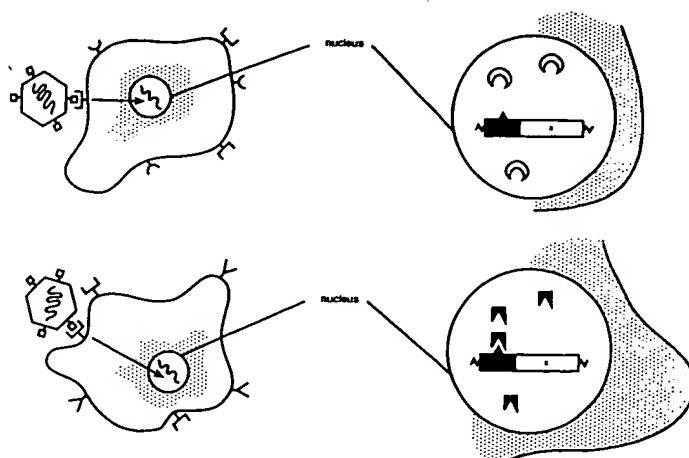


Figure 4. Tissue-restricted transcription. A promiscuously binding vector can be targeted at the transcriptional level if the therapeutic gene (x) is controlled by 5' regulatory elements (shown here as a shaded region upstream of x) active only in the presence of tissue-specific nuclear transcription factors; thus expression of x occurs only in the target cells.

Tissue-specific cellular regulatory elements have great potential for development of safe, targeted vectors for gene therapy. For example, the creatine kinase promoter has been used in a plasmid vector to restrict dystrophin cDNA expression to skeletal and cardiac muscle, and in the *mdx* mouse model of Duchenne muscular dystrophy, mice transgenic for this promoter-cDNA construct were found to exhibit correction of dystrophic symptoms (47). A potential approach to the treatment of B cell lymphoma involves expression of suicide genes transcriptionally regulated by promoter/enhancers from the Ig heavy chain, or the κ light chain genes; expression plasmids containing the diphtheria toxin A (DT-A) gene controlled by these regulatory elements mediated significant expression of DT-A in B lymphoid cells but not in HeLa cells or fibroblasts (48).

Endothelial cells are attractive recipients for gene transfer therapies not only for obvious purposes such as targeting of tumor vasculature or therapy of cardiovascular disease, but also for the systemic secretion of therapeutic factors. An endothelial cell-specific regulatory region has recently been characterized (49) as 500 bp of 5' sequences, associated with the gene for von Willebrand's factor, acting in conjunction with an essential region in the first intron. This promoter could be particularly useful when driving a suicide gene in a retroviral vector as it would then be targeted to dividing endothelial cells, i.e., almost exclusively tumor vasculature.

Tissue-specific cellular promoters frequently retain their specificity in the context of a retroviral vector (50); however, this is not always the case, and the design of the retroviral vector may have significant effects on tissue specificity due to promoter interference (51). Tissue-specific promoters have also been shown to appropriately restrict cDNA expression in the context of recombinant adenoviruses, e.g., the rat albumin promoter maintained its hepatoma cell specificity *in vitro* (52), albeit at low levels.

Antiviral therapy using transcriptional targeting

Transcriptional targeting may be of particular use in the therapy of particular kinds of viral infection. In cases where the viral life cycle depends on self-encoded autoregulatory

proteins, vectors can be made in which therapeutic cDNAs are transcriptionally regulated by these same viral proteins. Transcription of the therapeutic cDNA is therefore limited to cells that are infected by the virus, and thus such an approach could be either prophylactic or curative. This strategy has been applied to experimental HIV therapies. One recent report (53) described the construction of a recombinant retrovirus containing HSV-TK driven by the HIV-2 LTR-TAR; cells expressing this construct became susceptible to ganciclovir after infection by HIV-2 *in vitro*.

Targeting proliferating cells

Murine C-type retroviral vectors can combine the ability to express cDNA from an internal tissue-specific promoter with an innate tropism for proliferating tissue. Therefore, they have great potential as vectors for the gene therapy of cancer, because restricted cDNA expression is of particular importance in strategies that involve delivery of cytokine or suicide genes and malignancies are often distinguished by rapid division in a relatively quiescent background. Indeed, in a very few cases the retroviral requirement for cell division may be sufficient in itself to target the therapy (Fig. 5); where tumors arise in the CNS their high rate of proliferation in the context of a completely postmitotic tissue, in an anatomical compartment that is separated from the rest of the body, allows efficient targeting with retroviral vectors (54). As an additional targeting feature for malignancies of the CNS, the glial-specific promoter region of the mouse myelin basic protein gene has been used to drive HSV-TK in a retroviral vector (55); this approach could allow long-term administration of producer cells at the primary site or systemic vector appli-

cation to treat metastatic deposits as collateral infection of nonglial cells would not result in expression of the suicide gene.

Retroviral vectors would also be useful in targeting liver malignancies, as the liver is also slowly proliferative under normal circumstances. Tissue-specific promoters would be essential for such strategies, because unlike the CNS, the liver is not efficiently insulated from the rest of the body. Amphotropic retroviral vectors have been constructed carrying HSV-TK cDNA driven either by the albumin or the α -fetoprotein promoters (56). The albumin promoter was active only in cells of the liver lineage; the α -fetoprotein promoter conferred an extra level of targeting in that it was hepatoma-specific as opposed to hepatocyte-specific (α -fetoprotein is normally expressed only in fetal tissues).

The 5' region of the tyrosinase gene has also been used to restrict expression of therapeutic cDNAs to melanocytes and melanoma cells both *in vitro* and *in vivo* by means of retroviral vectors (51, 57). This kind of transcriptional targeting may be useful in VDEPT approaches for melanoma because normal melanocytes are dispersed and of low density in body tissues, and their ablation is likely to be minimally pathological. Even better would be the usurpation of tumor-specific transcriptional regulation by using promoter sequences from genes whose overexpression is limited to transformed tissue. One such candidate is the oncogene ERBB2, which is overexpressed in a variety of tumors. The ERBB2 promoter sequences have been used to drive cytosine deaminase cDNA in a retroviral vector (58); this strategy conferred sensitivity to ERBB2-overproducing cells but not to control cells, and represents a potentially widely applicable method of tumor-preferential transcriptional targeting. The α -fetoprotein promoter is in effect completely tumor-specific, but is applicable only to malignancies of the liver.

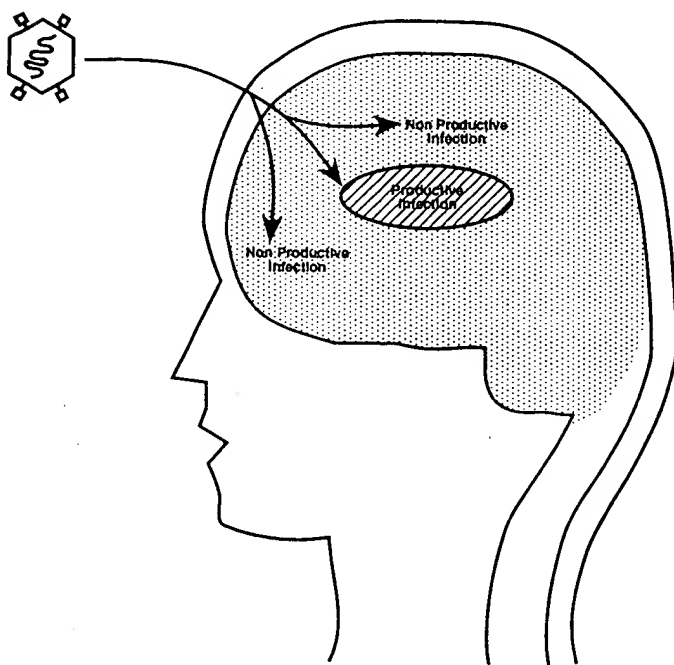


Figure 5. Targeting proliferating cells. Retroviral vectors require cell division for integration and gene expression; therefore where a tumor arises in a completely postmitotic background, such as the CNS, the proliferation of the malignant tissue may be sufficient in itself to allow efficiently targeted delivery of suicide genes via recombinant retroviruses. Actively replicating (tumor) cells are represented by diagonal lines; quiescent neuron tissue is represented by dots.

Exploitation of natural viral tropisms

An obvious approach to the precise targeting of tissues is to make vectors from viruses that have preferential patterns of transcription in target tissues, such as HSV vectors for nervous tissue. However, careful dissection of the genomes of these viruses will be necessary to separate pathogenic sequences from those that confer transcriptional specificity; in most cases it will be preferable to use cellular promoters in the vector of choice, especially as the range of transcriptionally targeted viral genomes is not great.

There may be one remarkable exception to the general requirement for cellular promoters rather than viral promoters in gene therapy, namely, the use of autonomous parvoviral sequences for targeting transformed cells (see ref 59 for review). These viruses preferentially kill transformed cells (60), and coinjection of mouse minute virus (MVM) and Ehrlich ascites tumor cells into the peritoneal cavities of mice inhibited tumor formation by up to 90%. Furthermore, mice that had survived one such coinjection were resistant to a second tumor challenge 5–6 wk later. The precise basis of parvovirus oncotropism is not understood but may be related to an effect of the transformed cell environment on the production or activity of parvovirus autoregulatory proteins. The parvovirus promoter that is preferentially transactivated in certain transformed cells is clearly a candidate to control transcription of suicide or cytokine genes in parvovirus vectors for cancer therapies. Recombinant parvovirus vectors have been made and shown to both transfer exogenous cDNA expression to recipient cells and retain their oncotropism *in vitro* (61) for human and murine cells. Recombinant parvoviruses may therefore represent one of the most promising approaches to cancer therapies for the future.

Targeted integration: site-specific recombination

Nonintegrating vectors are adequate for transient expression of cDNA. Where the object is a "one-shot" treatment for cure of a genetic disorder, it is necessary to use either an integrating vector or a stably replicating extrachromosomal element. For the future, sequences containing mammalian origins of replication or even entire mammalian artificial chromosomes (62) could have great potential especially for ex vivo approaches. Similarly, vectors based on the Epstein-Barr virus, which is stably maintained episomally as a plasmid in human cells, may one day be suitable for clinical use.

The ideal approach would be to target the exogenous DNA to the mutant gene, i.e., gene replacement rather than gene augmentation. Such gene targeting approaches may be of use for ex vivo strategies to stably transduce cells with less likelihood of simultaneous transformation (63). Such in vitro homologous recombination may be useful in inactivating genes responsible for MHC class I expression in myoblasts to create a universal carrier cell that can be transplanted regardless of the recipient HLA type (63). This approach is applicable to any ex vivo strategy that requires implantation of viable transduced but otherwise unchanged cells. The technology required to accomplish this at levels of efficiency relevant to in vivo gene transfer does not yet exist and so integrating gene therapy vectors at present can offer only gene augmentation.

Nontargeted integration could be hazardous if completely random, not only by turning on downstream oncogenes via promoter readthrough but also by direct disruption of genes, and this is the main source of concern with regard to the use of retroviral vectors in humans. Vectors with the capacity for site-specific integration would overcome these problems. Adeno-associated virus is a defective parvovirus that potentially is widely applicable in gene transfer strategies because it is tropic for many cell types, nonpathogenic in humans (in the absence of helper virus the AAV genome does not replicate but integrates into the genome and assumes a state of latency), and can be manipulated to derive recombinant genomes capable of vectoring exogenous DNA (64). Although these vectors can package only up to 4.5 kb as compared with the retrovirus limit of approximately 7 kb, they are said to have one major advantage over other integrating vectors, namely, a propensity (which is far from total) for apparently harmless integration into a region of human chromosome 19 known as AAVS1 (see review, ref 65). Where such specific integration occurs, it is almost certainly mediated by virally encoded proteins with affinity both for the target site and for the virus genome (66). Although integrated viral sequences remain dormant until superinfection by AdV/HSV, exogenous cDNAs driven by internal promoters can still be active (furthermore, the transcriptional inactivity of the viral ITR means that there will be no promoter interference leading to, for example, loss of tissue specificity of exogenous promoter, and less chance of insertional mutagenesis for the same reason). Thus AAV vectors have been shown to confer neomycin resistance and in some cases to integrate with site specificity (64). This study also showed that AAV vectors preserved their site specificity after transfection in plasmid form; the use of a transfectable plasmid rather than a viral vector might overcome the packaging limitations of AAV vectors (64). It must be said, however, that some groups report that recombinant AAV vectors show site specificity in only a relatively minor proportion of the total number of integration events. There have been several attempts to explore the therapeutic potential of AAV vectors, e.g., the delivery of cDNA for the correction of the cystic fibrosis defects (67).

There may be other vector systems also capable of site-specific integration. Eukaryotic genomes harbor large numbers of endogenous transposable elements of various types (68), i.e., autonomously replicating units that can insert themselves into the host genome. Some of these elements, known as LTR retrotransposons, are very similar to retroviruses both in replication cycle and in organization, being bound by LTRs and possessing coding regions with homology to retroviral *gag-pol* genes. The replicative cycle of LTR retrotransposons exactly parallels that of the retroviruses except that there is no envelope stage, thus, cytoplasmic virus-like particles (69) are formed containing reverse transcriptase, the RNA form of the retrotransposon, and cellular tRNA primers for reverse transcription. Such elements include *copia*, yeast Ty, and the intracisternal A particle of mice; clearly they have great potential as vectors of improved safety as their use with retroviral packaging lines would be less likely to result in helper virus production through homologous recombination. Indeed a mouse retrotransposon VL30 has already been made into a gene transfer vector (70), which can be produced in a standard retroviral packaging line. Endogenous retrotransposons a priori would be expected, through coevolution with the host genome, to display a degree of site specificity of integration as continuous random retrotransposition would be deleterious to the cell. Yeast retrotransposons offer the best examples of site-specific retrotransposons, and moreover, their site of integration appears to be benign. Two of the five *Saccharomyces cerevisiae* retrotransposons, Ty1 and Ty3, exhibit unambiguous site specificity of integration (71). Ty3 elements integrate into sites upstream of genes transcribed by RNA pol III, frequently within 1-4 nucleotides of the start site of transcription. It has been suggested that this sequence-independent site specificity is brought about by interaction of the retrotransposon with elements involved in RNA pol III-mediated transcription, e.g., TFIIB (71). Similarly, Ty1 preferentially integrates upstream of tRNA genes (71) 57% of insertions occurring within 400 bp of a tRNA gene. A consequence of this specificity is that yeast genes are only rarely interrupted by Ty1 insertions as regions upstream of yeast tRNA genes rarely contain open reading frames (71). The great similarity of LTR retrotransposons to retroviruses allows them to be made into vectors with conventional retrovirus packaging lines (70); possibly the development of a packaging line that provides retrotransposon rather than retroviral *gag-pol* in *trans* will allow the production of vectors with integrational site specificity.

SUMMARY AND PERSPECTIVES

Of the gene therapy protocols that have so far entered clinical trials, targeting of the appropriate vectors has been achieved largely only by indirect means. Thus, several such trials (for example, for treatment of ADA deficiency, HIV infection, or cancer) have used specific cell populations that have been removed from the patient and infected in vitro by nontargeted amphotropic retroviruses before being returned in vivo. Further levels of targeting have been achieved in some cases by careful choice of the patient's cells; for instance, ex vivo transduction of tumor infiltrating lymphocytes with potentially tumoricidal genes has been proposed as a means of delivering their products to tumor deposits at much higher concentrations than would otherwise be possible.

In contrast to ex vivo manipulation of target cells where the vector requires very little, if any, intrinsic targeting capability, there are an increasing number of protocols in which

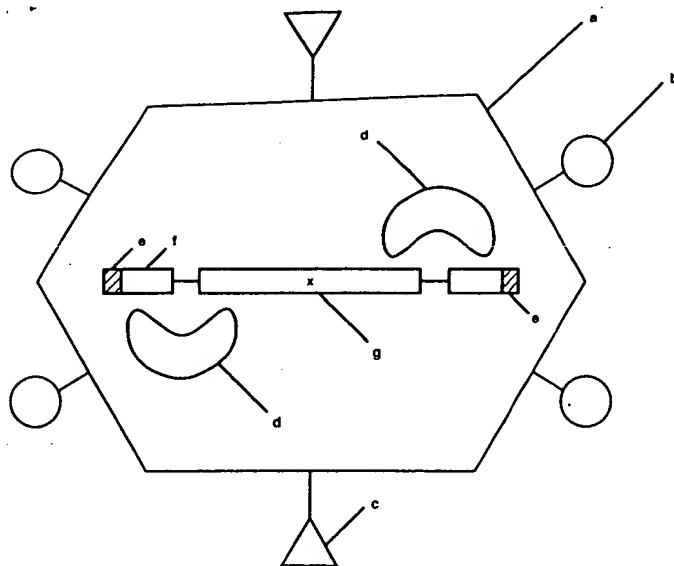


Figure 6. A theoretical composite vector. Some features that might be incorporated in an ideal synthetic vector include a stable, nonimmunogenic envelope, probably lipid (a); exposed ligands to confer a particular affinity on the vector (b); moieties that encourage fusion between vector and target cell membranes (c); proteins to allow directed integration of vector DNA, e.g., site-specific recombinases (d); sequences to enable homologous recombination between vector DNA and particular loci of the target genome (e); tissue-specific promoter regions to allow restricted expression of the therapeutic gene (f); and the therapeutic cDNA (g).

recombinant genes are delivered directly to patients in vivo (such as for the treatment of cystic fibrosis and cancer). Once again, targeting at the level of the vector has not yet been particularly well developed; hence, liposome or viral-mediated delivery of the CFTR gene to airway epithelial cells of CF patients has relied largely on the localized delivery of the vectors directly to the affected tissues, and on the fact that there is good evidence that inadvertent expression of the CFTR gene in cells other than the target epithelial cells may have few adverse effects. Localized delivery has also been used in the treatment of brain tumor deposits, using stereotactic injection of retroviral producer cells, but with the added sophistication that the retroviruses would be expected to infect only the actively dividing tumor cells and not the surrounding neural tissue.

However, for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances in the ability with which clinicians can confidently administer recombinant vectors for the treatment of genetic disease directly to affected tissues in vivo. For this to occur, many targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems. Vectors have already been developed that incorporate transcriptional specificity for a certain tissue type; however, the development of surface targeting has been more problematic in most cases. The biggest challenge for the next 5 years will be to combine targeting with efficiency in the production of the vector systems of the future. So far, attainment of one usually compromises the other; for example, we have constructed retroviral vectors targeted at the level of transcription to melanoma cells but these viruses are generally of lower titer than their nontargeted counterparts.

Nonetheless, the imagination and the technology is currently available to allow us to hope that vectors will eventually be constructed that can include both efficiency and specificity. In particular, it does not seem unrealistic to suppose that the gene therapy vectors of the future will not be based exclusively on any single virus or physical vector system alone but will be synthetic, custom-designed vehicles (Fig. 6) into which specific targeting features can be included depending on the particular clinical requirements of the target disease and tissue.

[F]

We would like to thank Professor Bob Williamson for influential discussions.

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Relation of *Campylobacter pyloridis* to Gastritis and Peptic Ulcer

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Biopsy specimens from the gastric antral mucosa of 50 patients with upper gastrointestinal complaints were studied by light and electron microscopy and culture. Of 46 assessable specimens, seven were histologically normal, and 39 showed evidence of gastritis. Twenty-seven of the specimens with evidence of gastritis (69%) contained spiral bacteria, whereas only one of the normal specimens (14%) contained these bacteria ($P = .02$). Of 17 patients found to have gastric ulcers, 10 (59% [$P > .10$]) also had spiral bacteria. The bacteria could be seen scattered over the surface of the epithelial cells and just under the layer of mucus but were rarely found inside the epithelial cells. Curved or spiral gram-negative bacilli were isolated from 10 of the specimens on chocolate agar incubated at 37 C under microaerophilic conditions. The bacteria resembled the organism recently named *Campylobacter pyloridis* by other investigators.

Gastritis and peptic ulcers are major conditions that affect numerous individuals every year. The etiology and pathogenesis of these conditions are not fully understood at present. The term *gastritis* includes a group of inflammatory states of the stomach differentiated according to the area affected, extent of damage, and type of inflammatory cell present [1]. The etiology is known in some of these conditions, but in others it is less well defined. The physiological basis of peptic ulcers is not entirely clear, but it is thought to be related to the relative balance of acid production to the resistance of mucous lining covering the gastric epithelium. Stress and other unknown factors are thought to affect this relative balance [2].

Recently, Marshall and Warren [3] and Warren and Marshall [4] presented evidence that a new species of *Campylobacter*, named *Campylobacter pyloridis* [5], was associated with gastritis and peptic ulcers; it was suggested that this organism might participate in causing these diseases. Other preliminary reports have also appeared with similar results [6-9]. The work reported here was initiated to confirm this observation and to explore further the characteristics of this organism.

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Informed consent was obtained from all patients participating in this study.

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Materials and Methods

Patients studied. A total of 50 consecutive patients presenting to the Gastroenterology Clinic at the University of Texas Medical Branch (Galveston, Tex) was studied. The age range of the patients was 22-77 years, and the median age was 50 years. There were 25 men and 25 women. The endoscopy procedure was performed to investigate abdominal symptoms such as epigastric pain, nausea, vomiting, rectal bleeding, etc. Five of the patients were receiving antibiotics at the time of endoscopy: two trimethoprim/sulfamethoxazole, one erythromycin, one cephadrine, and one ampicillin. Any individual with coagulopathy or requiring emergency endoscopy was automatically excluded from the study. With each patient the gastric antrum was visualized by endoscopy, and in addition to a routine biopsy specimen taken for routine diagnosis, several additional pieces of tissue, ~1 mm in diameter, were excised.

Light microscopy. After the specimens were obtained, one piece was fixed in 10% buffered formalin for light microscopy, another was saved for electron microscopy by fixation with 2% glutaraldehyde in 0.2 M cacodylate buffer for 2 hr and then stored in the same buffer at 4 C until processed, and a third piece was cultured. The formalin-fixed specimens were embedded in paraffin with use of standard procedures, sectioned, and stained with hematoxylin and eosin, gram stain, and Dieterli's silver stain. The slides were coded with a random number and examined in a blind manner to prevent bias. Each slide was examined independently by two patholo-

gists (W.K.L. and W.K.G.), who then reconciled any differences before the results were correlated. The diagnosis of gastritis was based on the amount and character of inflammatory infiltrate in the lamina propria or gastric gland. The absence or presence of intestinal metaplasia was noted. Categorization was not difficult when acute inflammation of the tissue was present. When such inflammation was absent, assessment of gastritis was more subjective and difficult to evaluate consistently. Therefore the cases were classified into three categories: normal, clear-cut gastritis, and borderline chronic gastritis. The last term was used when the only abnormality was an increased number of lymphocytes and plasma cells in the lamina propria, when compared with specimens classified as normal.

Electron microscopy. The specimens were post-fixed in 1% osmic acid, washed, dehydrated in a graded series of ethanol, and embedded in epoxy plastic. Plastic-embedded sections 1- μ m thick were stained with methylene blue-azure II and basic fuchsin [10].

Isolation and characterization of bacteria. Biopsy specimens were inoculated onto chocolate agar plates made with GC medium base supplemented with 0.025% yeast extract, 1% hemoglobin, and 1% V-enrichment (Austin Biological Laboratories, Austin, Tex). The plates were incubated in an atmosphere of 5% O₂, 10% CO₂, and 85% N₂ at 37 C and examined daily for seven days. The atmosphere was generated by placing the plates in a torbal jar (American Scientific, Houston), evacuating the jar with the laboratory vacuum line, and refilling the jar with a commercially prepared gas mixture (Linde Division, Union Carbide, Somerset, NJ). A square of absorbent paper containing several drops of tap water was included in the jar to supply additional moisture.

Biochemical characteristics of the isolates were determined as follows. Oxidase and catalase tests were performed in the standard manner [11]. Glucose fermentation was determined with purple broth base containing 1% glucose. Production of H₂S was determined with use of triple sugar iron agar. Indole production was determined by incubation of the isolates in tryptone broth, extraction of the broth with xylene, and addition of Erlich's reagent [11]. Reduction of nitrate was determined with use of nitrate broth. Hippurate hydrolysis was tested by the method described by Lior [12]. Susceptibility to nalidixic acid and cephalothin were tested with use of 30- μ g disks (General Diagnostics, Santurce, Puerto Rico) on

Columbia blood agar base. Where necessary, the media were supplemented with horse serum at a final concentration of 5%, except in the case of agar slants, where a few drops were added to the slant.

Growth at 25, 37, and 42 C was assessed by inoculation of the isolates onto chocolate agar plates and incubation of the plates in a commercially supplied plastic bag with a gas-generating vial (Bio-Bag environmental chamber, type cfj; Marion Laboratories, Kansas City, Mo) at these temperatures. To determine whether the isolates grew anaerobically, we streaked them onto chocolate agar and incubated them at 37 C in a similar plastic bag that generates an anaerobic atmosphere (Bio-Bag, type A, Marion Laboratories). A similar plate was also incubated in ambient air at 37 C. Additional growth characteristics of several isolates were examined by inoculating them to various media that were plain (without supplementation) or with blood or serum supplements. Three to seven isolates were streaked onto the plates, and the plates were incubated in a microaerophilic atmosphere at 37 C for three days.

Isolates were tested for antibiotic susceptibility by agar dilution [13] on Mueller-Hinton agar containing 10% horse serum and incubation in a microaerophilic atmosphere at 37 C. Two concentrations of each antimicrobial agent, corresponding to the breakpoint concentrations, were tested. The following antimicrobial agents and concentrations were tested: vancomycin, 5 and 10 μ g/ml; erythromycin, 4 and 8 μ g/ml; chloramphenicol, 12.5 and 25 μ g/ml; clindamycin, 1 and 2 μ g/ml; cephalothin, 8 and 16 μ g/ml; gentamicin, 4 and 8 μ g/ml; and tetracycline, 6 and 12 μ g/ml.

Statistical methods. Statistical analyses were

Table 1. Correlation between inflammation in antral biopsy specimens and the presence of spiral bacteria.

Histological category	No. of patients			p
	Total	Spiral bacteria		
		Positive	Negative	
Normal	7	1	6	...
Borderline gastritis	11	9	2	.02
Gastritis	20	15	5	.02
Gastritis with				
intestinal metaplasia	8	3	5	.67
Total with gastritis	39	27	12	.02
Total	46	28	18	...

* In comparison with normal tissue.

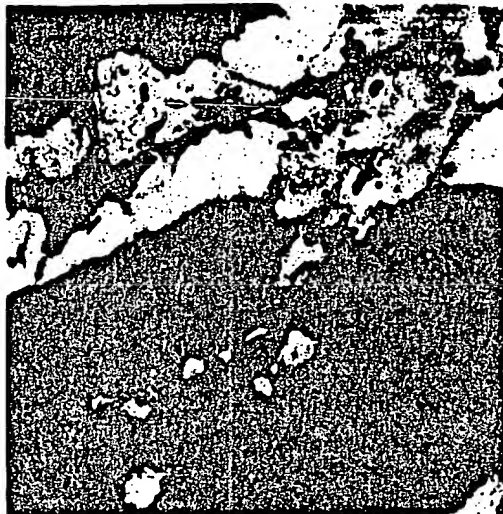


Figure 1. Histological section of a biopsy specimen from the gastric antrum stained with Dieterli's silver stain ($\times 950$).

done with the χ^2 test with Yates's correction for continuity.

Results

Of the 50 patients evaluated, 4 were excluded because their biopsy specimens were too small for adequate



Figure 2. Gram-stained smear from a biopsy specimen shows numerous spiral or curved bacteria ($\times 950$).



Figure 3. Electron micrograph of the luminal surface of a gastric foveolar crypt. Transversely and longitudinally sectioned spiral bacilli (arrows) are adjacent to the surface of mucous epithelial cells. Bar = 1 μ m.

evaluation, 7 were found by histological examination to have normal gastric mucosa, and the remaining 39 patients had some degree of gastritis. Spiral bacilli were found in 27 (69%) of these 39 patients and in only one (14%) of the seven patients with histologically normal gastric mucosa ($P = .02$). The presence of bacilli and category of inflammation are compared in table 1. A total of 17 (37%) of the 46

Table 2. Growth characteristics of strains of *Campylobacter* isolated from human gastric tissue.

Isolate no.	Growth in			Growth at		
	Ambient air	Anaerobic atmosphere	5% CO ₂	25 C	37 C	42 C
2	-	±	+	-	+	+
5	-	-	+	-	+	+
6	-	-	+	-	+	+
7	-	±	+	-	+	+
10	-	±	±	-	+	+
11	-	-	±	-	+	+
16	-	+	+	-	+	+
17	-	-	+	-	+	+
18	-	+	+	-	+	+
19	-	+	+	-	+	-

patients had a gastric ulcer or erosion visible on endoscopic examination, and spiral bacilli were found in 10 (59%). There was no significant difference in the frequency of bacilli in patients with ulcer or erosion compared with patients with histological gastritis ($P > .10$). There was also no significant difference between patients with ulcer and those with histologically normal mucosa. Of the five patients who were receiving antibiotics at the time of the study, the organism was either isolated or seen in tissue from three, and tissue from the other two was negative. None of the patients with normal histology was receiving antibiotics.

The bacteria could be seen most easily in tissue sections with silver stain (figure 1), but they could also be seen with Brown-Brenn tissue gram stain or even with the routine hematoxylin and eosin stain. They occurred on the surface of the epithelial cells and in the lumen, under the mucous layer. In many cases the bacteria could be readily demonstrated in a gram-stained smear directly from the tissue (figure 2). Examination of the tissues by electron microscopy (figure 3) confirmed that the bacteria occurred primarily in close association with the surface of the epithelial cells.

Curved, gram-negative bacilli were isolated from 19 of the specimens. The isolates grew slowly, requiring four to seven days for recovery in culture, but on subculture the colonies reached maximal size in about three days. The colonies were circular, convex, translucent, and nonpigmented, and the maximal size was ~0.5 mm. All isolates were oxidase and catalase positive. Staining of flagella by the Ryu method [14] showed that the cells had a tuft of polar flagella. Ten of the isolates were characterized

further. Growth in different atmospheric conditions or at 25, 37, or 42 C is shown in table 2. None of the strains fermented glucose, hydrolyzed hippurate, or produced indole or H₂S. Two of the strains reduced nitrate, but the others were negative. All were resistant to nalidixic acid and susceptible to cephalothin by the disk diffusion test. These characteristics fit those previously published for *C. pyloridis* [3-5]. A unique feature of these organisms was that the morphology in pure culture differed slightly from that seen in tissue. In pure culture (figure 4) the bacteria were frequently more rodlike, and bizarre shapes, such as U-shaped cells or circular cells, could be seen. Eight of the isolates were further examined for susceptibility to common antimicrobial agents with use of agar dilution methodology and standard breakpoints for interpretation [12]. All of these isolates were resistant to vancomycin (MIC, $<1 \mu\text{g/ml}$) but susceptible to erythromycin (MIC, $<4 \mu\text{g/ml}$), clindamycin (MIC, $<1 \mu\text{g/ml}$), cephalothin (MIC, $<8 \mu\text{g/ml}$), gentamicin (MIC, $<4 \mu\text{g/ml}$), and tetracycline (MIC, $<6 \text{ mg/ml}$).

An experiment (table 3) was performed to investigate the growth on various media and the requirement for blood or serum. Three to seven isolates were streaked on the various media, and growth was assessed after three days at 37 C under microaerophilic

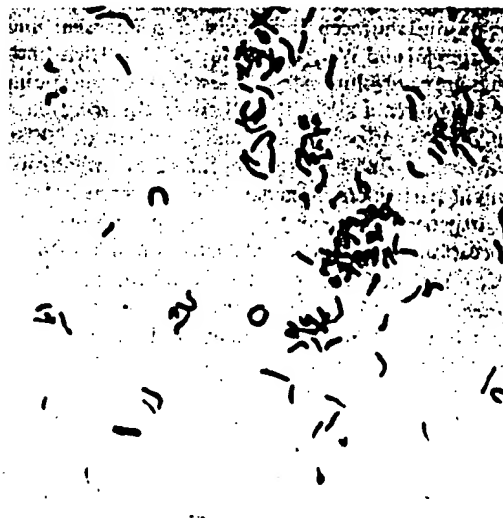


Figure 4. Gram-stained preparation from a colony of bacteria isolated from a gastric biopsy specimen. The cells are more loosely curved and exhibit unusual morphological forms ($\times 950$).

Table 3. Growth of representative isolates on various media.

Medium	No supplement	5% serum		5% blood	
		Horse	Sheep	Rabbit	Sheep
Chocolate	+	ND	ND	ND	ND
Thayer-Martin	+	ND	ND	ND	ND
GC agar	+	+	+	+	+
Columbia	0	+	+	+	+
Trypticase soy	0	+	+	+	+
Brain-heart infusion	0	+	+	+	+
Brucella	0	+	+	+	+
Nutrient agar	0	0	0	0	0

NOTE. Growth was rated on the following scale: + = growth; 0 = no growth. ND = not done.

conditions. All of the isolates tested grew on GC agar base without any supplement but not on the other media tested unless blood or serum was present. No growth was obtained with use of nutrient agar, even with blood or serum supplementation.

Discussion

Spiral bacteria were originally observed in human gastric tissue many years ago [15], but early investigations to confirm this observation and to examine its significance yielded conflicting results [16, 17]. Therefore these observations were ignored for many years until the recent investigations of Marshall and Warren [3] and Warren and Marshall [4]. Their findings are of revolutionary interest because they point to a significant association between the occurrence of gastritis and peptic ulcers and the presence of these bacteria, a finding implying that these organisms might participate in causing these diseases.

In general, our results are consistent with their observations. We found spiral bacteria in only 1 (14%) of 7 patients with histologically normal gastric mucosa but in 27 (69%) of 39 patients with gastritis and 10 (59%) of 17 of patients with gastric ulcer. Statistical analysis showed a significant difference between the normal group and patients with gastritis but not between the normal group and patients with ulcer. Therefore our results indicate a distinct association of the bacteria with gastritis; this conclusion is in agreement with other recent findings [5-9]. Our results failed to show a definite association with ulcers. This conclusion agrees with the results of Rollason et al. [6] and Burnett et al. [9] but conflicts with the results of other investigations

[5, 7, 8]. As larger numbers of patients are studied, these conflicts may well resolve.

Because the patients examined were consecutive and essentially unselected, some of them were receiving antibiotics at the time of examination. Antibiotic use apparently had minimal effect on the results because three of these patients were positive for bacteria, and two were negative. None of these patients was in the group with normal histological findings.

The fact that these organisms can be found in electron micrographs in large numbers and in close association with the surface of epithelial cells deep in foveolar crypts suggests they are not merely mouth flora that contaminated the biopsy specimens on withdrawal of the endoscope. However, although these bacteria are clearly associated with gastritis and probably also associated with gastric ulcers, their significance as far as causing these diseases is still in question. In this regard we have preliminary information (authors' unpublished observation) that inflammatory cells are present in the gastric lumen ingesting these bacteria, thus suggesting an active effort on the part of the host to remove these bacteria.

The organism recovered from the tissues has unique characteristics. It is oxidase and catalase positive and grows either under microaerophilic conditions or in 5% CO₂ but not in ambient air, and most isolates do not grow under strict anaerobic conditions. A unique characteristic is its change in morphology when grown in vitro. Because of this alteration we initially wondered whether this organism really was the same as that seen in tissues. However, the isolates were found in high numbers in many of the specimens and were not found in routine cultures of the mouth from 20 randomly selected individuals (authors' unpublished observation). Therefore we believe the organisms isolated are the same as those seen in tissue, although this conclusion cannot be proved at the moment. One means of substantiating this proposal would be to make antibody to the isolates and determine whether the antibody reacts with spiral bacilli found in tissue sections. Also, preliminary comparison of our isolates with several strains supplied by Dr. B. J. Marshall shows that they are similar in characteristics.

Although this organism has recently been named *C. pyloridis*, there are several characteristics that do not really fit the genus *Campylobacter*. First, the bacteria have multiple polar flagella rather than a single flagellum, and, in addition, according to Marshall and Warren [3], the flagella are sheathed.

Second, the unusual morphology in pure culture is different from any other species of *Campylobacter* known. Third, the failure to grow on most media without added serum is a feature also not found in the other *Campylobacter* spp. These characteristics are sufficiently different from known *Campylobacter* spp. that this organism may actually represent a new genus. Genetic characterization would settle this question.

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